CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Chemical name:

metam-sodium (ISO); sodium methyldithiocarbamate [1]; and

metam-potassium (ISO); potassium methyldithiocarbamate [2]

EC Number: Group of substances: 205-632-2
[1] 205-293-0
[2] 205-292-5

CAS Number: Group of substances:144-54-7
[1] 137-42-8
[2] 137-41-7

Index Number: /

Contact details for dossier submitter:

FPS Public Health, Food Chain Safety and Environment
DG EM/ Department of Product Policy and chemical Substances / Management of Chemical Substances
BELGIUM

On behalf of the Belgian Pesticides unit

Version number: 3 **Date: May 2023**

European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

METAM

Volume 1

Rapporteur Member State: Belgium
Co-Rapporteur Member State: Spain

Version History

When	What
August 2007	Initial Draft Assessment Report (DAR) – prepared by RMS BE in the context of the EU review of metam as existing active substance, in accordance with Council Directive 91/414/EEC, with a view to the possible inclusion of the substance in Annex I to the Directive. Addenda and updates to the initial DAR were issued in June 2008.
August 2010	Revised Draft Assessment Report (DAR) – prepared by RMS BE in the context of the application for inclusion of the active substance in Annex I to Council Directive 91/414/EEC, in accordance with the accelerated procedure foreseen under Reg. (EC) No 33/2008, following non-inclusion. Addenda to the revised DAR were issued in November 2010 and April 2011 and were compiled by EFSA in a final 'addendum' dated May 2011.
July 2021	Draft Renewal Assessment Report (DRAR) – prepared by RMS BE in the context of the application for renewal of approval of the active substance according to Reg. (EU) No 844/2012.
April 2022 March 2023	Amendments consecutive to the recommendations and requirements of ECHA for the CLH accordance check
June 2023	Amendments consecutive to the recommendations and requirements of ECHA for the CLH accordance check of Metam (2nd sending)

Note 03/2023:-in order to meet the desire of ECHA, RMS splitted in two the volume 1, in order to allow a more convenient evaluation of the CLH for Metam and its main metabolite MITC.
This volume contains the CLH of Metam-sodium (parts regarding CLH of MITC are greyed out). Proposed harmonised classification and labelling of MITC can be found in section 2.11.

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Level 1

METAM

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

The Draft Renewal Assessment Report (DRAR) has been prepared in the framework of the evaluation of the application for **renewal of the approval** of metam according to Reg. (EU) No 844/2012. Metam is part of the AIR-5 programme. A proposal for **MRL setting** is not included.

A fully revised proposal for **Classification & Labelling** is included.

Important remark regarding practical arrangements as regards the hazard assessment (C&L, ECHA) *vs.* risk assessment (EFSA), for whom it may concern.

In line with the current safety assessment procedure, the CLH was embedded in Vol.1 of the a.s. metam. The purpose is to harmonise the assessment by the two expert groups.

Two points of attention are to be highlighted: , especially to fulfill the desires of ECHA as regards the CLH and concomitant opinion of the RAC.

1. Issue of the **variants** of metam

As extensively explained below, metam exists in 2 variants: the sodium- and the potassium salt, each forming the metam acid, named methyldithiocarbamic acid. During the peer review of the first approval of the a.s., it has been agreed that both variants were equivalent from the point of view of safety (human toxicity, ecotoxicity and ecochemistry). It seems straightforward and common sense that counter-ions like sodium or potassium are unlikely to influence in a meaningful way the toxicity or the environmental fate of the acid.
Whereas most of the safety studies have been performed with the metam sodium variant, it has been, and it is still scientifically justifiable to consider that the database covers both hazard and risk assessment of metam overall.
Existing bridging studies (mostly acute studies, performed with the two variants) confirm that both the sodium- and potassium variants have comparable (eco)toxicological profiles, and hence, should be classified and labelled the same.
Based on the Tier II equivalence assessment, both variants are considered to be equivalent (please refer to the confidential DAR, Vol. 4 for further details). The variant metam-sodium is considered to be the **reference source**.
The active substance variants, metam-sodium and metam-potassium are produced as technical concentrates (TK) containing 510 g/L metam-sodium or 690 g/L metam-potassium, respectively. The manufacturing process for the active substance is such that the technical concentrate (TK) is identical to the commercial plant protection product.
The amendments of the entries in Annex VI are thus made accordingly.

2. Issue of the **metabolites** of metam

Metam is a liquid, which both in the environment and in biological matrices immediately and completely degrades (biotically and abiotically) in a number of metabolites. The main metabolite however is methyl isothiocyanate (a.k.a. MITC), which is released as a gas and constitutes as such the ultimate active substance (a.s.). This metabolite is responsible for the biological activity and efficacy of the fumigant. It follows that humans and non-target organisms will ultimately be exposed to MITC, and that the latter thus –in practice– drives both the hazard and risk assessment of the a.s. and the PPP, at least in the context of the currently proposed GAP.

It may thus to a certain degree be reasonably expected that the release of MITC will drive the toxicity of metam. However, taking into account the different routes of exposure (MITC being a gas, metam being a liquid), both toxicokinetic and toxicodynamic differences may lead to some differences in toxicological profile of metam and MITC. In addition, minor breakdown products of metam (including instable intermediates) may be formed (MIC, methylamine, COS, H_2S, CS_2), which may also be degradates of MITC itself. Consequently, different entries of metam and MITC are necessary, also because MITC may also be present on the market as a separate substance, also in different physical form (liquid, solid).

In any case, the hazard and risk assessment of metam cannot entirely be dissociated from that of its main metabolite MITC, and are therefore considered in parallel in this EU-dossier. A same approach has been followed for other

fumigants like Dazomet, which also releases MITC and minor breakdown products as the ultimate «active» metabolite, albeit following a slightly different degradation kinetics.

In general, it is a common practice to consider together the a.s. and its metabolites, both in cases where these metabolites are less toxic (detoxification) and more toxic (toxification).

For reasons of organisational and procedural order, ECHA considered however difficult to maintain this approach, because, as also indicated above, MITC is at this moment a separate entry in Annex VI of Reg. (EC) no 1272/2008[i]. For this reason, RMS split Vol.1 in two parts, in order to allow ECHA to «separately» initiate the discussion on metam and MITC, and to avoid insurmountable practical inconveniences during their peer review, public consultation and publication on their website. The two parts destinated to ECHA therefore contain greyed out texts of metam for the MITC sub-part, and *vice-versa* of MITC for the metam sub-part.

For the Peer review under the PPP-regulation, both parts, including amendments and comments of the RAC, can be considered and easily fused again when the Peer Review of the a.s. under Reg.(EC) no 1107/2009 is initiated.

During accordancy check, ECHA added a request that an assessment should be made for each hazard class (human health and environment) as to whether (the classification of) MITC is relevant for classification of metam, requesting RMS to apply it for any hazard class in the CLH dossier.
RMS is of the opinion that, whatever the classification of the main metabolite would be, a theoretical approach as suggested by ECHA is not necessary. We observe that for all endpoints, metam itself has been tested, and the existing assays are relied upon and considered sufficient for the sake of classification and labelling in all relevant sections of physical chemistry, toxicology, environmental fate, and ecotoxicology. Conversely, the same holds for MITC: each endpoint is covered by the tests performed with MITC.
Introducing at this stage considerations of time-related release of methylisothiocyanate (MITC) in the tested indicator-organism or any other substrate as a key factor in the establishment of C&L of metam is not useful. The a.s. metam is known to be degraded to a.o. MITC (see toxicokinetics studies) in more or less the same way as it is degraded in the environment. In other words, any (eco)toxicological effect is influenced by the biodegradation to all its metabolites (including MITC), impacting on the overall (eco)toxicity of metam.
In conclusion, subsequent to ECHA's remark, RMS considers it sufficient to state that, while MITC may be of lower or greater toxicity than the metam salts for certain endpoints, the C&L of the latter will not determine in a 1-to-1 way the C&L of metam itself. Toxicokinetic studies in vertebrates have demonstrated that the oral absorption of metam and its major metabolite MITC is rapid and complete, reaching a mean value of 85%, based on its excretion in urine (50%) and expired air (35%) for metam, and 90-95% for MITC, based on its excretion in urine (80-85%) and expired air (10-15%). Half-life of elimination from plasma are in the order of magnitude of about 3 days.
The formal classification of MITC is not considered to be per se more relevant for the assessment of any hazard class of metam, although it is acknowledged that obviously (as indicated in the DAR), it is expected an important metabolite driving the (eco) toxicity of its carrier metam. Therefore, RMS considers that data for metam salts (Na or K) are used to classify for any hazard class, in the same way as the studies are usable to derive reference values for the (eco)toxicological risk assessment.

For a good understanding, when RMS remarked that hazard and risk assessment of metam cannot entirely be dissociated from that of its main metabolite MITC, and should therefore considered in parallel in this EU-dossier, it was to emphasise its practical consequence for the risk assessment in vol.1 of the DAR. A same approach has been followed for other fumigants like Dazomet, which also releases MITC and minor breakdown products as the ultimate «active» metabolite, albeit following a slightly different degradation kinetics.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

The RMS is Belgium (BE), the co-RMS is Spain (ES). Before submission of the DRAR to the EFSA, draft versions have been provided to ES for commenting, at least for the sections Identity, Physical and chemical properties, Methods of analysis, and Fate and behaviour. Comments have been taken into account and agreements or disagreements have been indicated in the DRAR, where relevant.
With regard to the parts dealing with human health, residues and ecotoxicology, a consultation of the co-RMS was not possible before submission to EFSA; the co-RMS will provide its comments during the official commenting period of the peer review open to all MSs.

1.1.3 EU Regulatory history for use in Plant Protection Products

Metam is a substance that was covered by the 3rd stage of the work programme for review of existing active

substances provided for in Article 8(2) of Directive 91/414/EEC concerning the placing of plant protection products on the market1, with a view to the possible inclusion of this substance in Annex I to the Directive.
At the outcome of that evaluation, metam was not included through Council Decision 2009/562/EC, due, in particular, to data gaps in the consumer risk assessment and fate and behaviour of the major metabolite MITC and impurity DMTU, to concern for potential contamination of groundwater and for operators and workers in glasshouses applications. All information as regards this initial evaluation is recorded in the relevant Commission Review Report (document SANCO/206/2008 final of 28 July 2009).
In accordance with Article 13 of Regulation (EC) No 33/20083, Taminco N.V., at that time the sole data submitter presented, on 12 January 2010 a request to Belgium, the original rapporteur Member State, for a new application aiming at Annex I inclusion of the substance.
Belgium finalised in August 2010 its examination, in the form of an additional report to the original Draft Assessment Report. This Report was received by the Commission and the European Food Safety Authority on 31 August 2010 and included a recommendation as to include metam in Annex I for the supported uses.
The EFSA organised the consultation on the draft assessment report and, in accordance with the provisions of Article 19 of Regulation (EC) No 33/2008, on the additional report by all the Member States as well as Taminco N.V. being the sole data submitter, on 14 September 2010 by making it available.
The EFSA organised a focused consultation of scientific experts in the areas of toxicology, environmental fate and behaviour and eco-toxicology from a certain number of Member States, to review the additional report and the comments received thereon (peer review).
In accordance with the provisions of Article 20 of Regulation (EC) No 33/2008 the EFSA sent to the Commission its conclusion on the risk assessment. This conclusion refers to background document A (draft assessment report and additional report) and background document B (EFSA peer review report).
In accordance with the provisions of Article 21 of Regulation (EC) No 33/2008, the Commission referred a draft review report to the Standing Committee on the Food Chain and Animal Health, for final examination. The draft review report was finalised in the meeting of the Standing Committee on 9 March 2012.

1.1.4 Evaluations carried out under other regulatory contexts

- Existing **EU MRLs** for metam (and dazomet, another MITC-generating substance) were fully reviewed by EFSA in the framework of art.12 of **Reg. (EC) No 396/2005** (EFSA, 2019a; EFSA, 2019b), which led to the agreement by the European Commission and Member Statement (at the SCoPAFF Residues meeting of February 2021) on revised EU MRLs for MITC for inclusion in Annex II of Reg. (EC) No 396/2005. At the moment of finalising the DRAR, the regulation establishing these agreed revised EU MRLs had not yet been published.
- At the level of FAO/WHO, metam has not been evaluated by the **JMPR** (Joint FAO/WHO Meeting on Pesticide Residues), but metam-sodium has been evaluated by **JMPS** (Joint Meeting on Pesticide Specifications)[1].

Other, most recent evaluations outside the EU include:
- Metam Sodium (Sodium N-Methyldithiocarbamate), risk characterization document, Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency, July, 2004.
- U.S. Environmental Protection Agency (Amended Reregistration Eligibility Decision (RED) for the Methyldithiocarbamate Salts (Metam-sodium, Metam-potassium) and Methyl Isothiocyanate (MITC). Prevention, Pesticides and Toxic Substances. May 2009.
- Chemical meeting the criteria for listing as causing Cancer via the authoritative bodies mechanism: Metam potassium PACKAGE 34b, Reproductive and Cancer Hazard Assessment Branch Office of Environmental Health Hazard Assessment California Environmental Protection Agency, February 2010.
- Metam-sodium is registered at ECHA, as chemical with a total tonnage band in the range: ≥1 to <10 tonnes/year, although no chemical safety assessment is foreseen, April 2018.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Two individual dossiers were submitted for this active substance.

Applicant 1 – Taminco BV:

[1] http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Old_specs/Meta.doc

Note: In accordance with the new Belgian Code on Companies and Associations, Taminco BVBA's legal form was formally converted into Taminco BV. Other than legal form change, all other details of the company as well as its address will remain unchanged.

Company	Taminco B.V, a subsidiary of the Eastman Chemical Company
Address:	Pantserschipstraat 207, B-9000 Gent Belgium
Contact:	[redacted]
Telephone:	[redacted]
Fax:	not applicable
Email:	[redacted]

Primary Contact:

Company:	Knoell Germany GmbH **On behalf of Taminco BV, a subsidiary of the Eastman Chemical Company**
Address:	Konrad-Zuse-Ring 25, 68163 Mannheim. Germany
Contact:	[redacted]
Telephone:	[redacted]
Fax:	Not applicable
Email:	metam@knoell.com

Applicant 2 – Lainco S.A.

Company:	Lainco, S.A.
Address:	Poligono Can Jardi, Avda. Bizet, 8-12, 08191 Rubi, Barcelona, Spain
Telephone:	+34 935862015
Fax:	+34 935862016
Contact person:	[redacted]
Telephone:	[redacted]
Fax:	[redacted]
Email:	[redacted]

1.2.2 Producer or producers of the active substance

Applicant 1 – Taminco BV

Company	Taminco BV, a subsidiary of the Eastman Chemical Company
Address:	Pantserschipstraat 207, B-9000 Gent Belgium
Contact:	[redacted]
Telephone:	[redacted]
Fax:	not applicable
Email:	[redacted]

Applicant 2 – Lainco S.A.

Company:	Lainco, S.A.
Address:	Poligono Can Jardi, Avda. Bizet, 8-12, 08191 Rubi, Barcelona,

Spain
Telephone: +34 935862015
Fax: +34 935862016

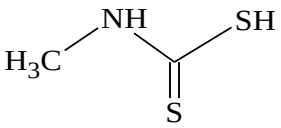
Production sites are confidential information and available in the respective Vol. 4.

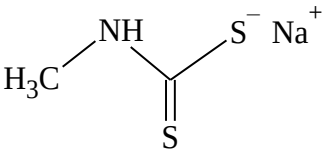
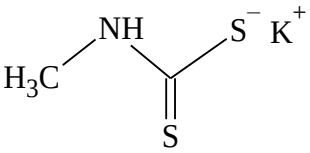
1.2.3 Information relating to the collective provision of dossiers

Two individual dossiers were submitted: Applicant 1 – Taminco BV and Applicant 2 – Lainco SA

For the renewal of approval of metam (incl. -potassium- and -sodium), in addition to Taminco BV, an application was submitted by Lainco SA. Although both companies have been in contact and discussed the possibility to submit a joint dossier, no agreement could be reached. Pursuant to Articles 61 and 62 of Regulation (EC) No. 1107/2009, an agreement to share studies ('Memorandum of Understanding'), expert statements and consultancy works has been reached between Taminco BV and Lainco SA, avoiding duplicative testing/conclusions. The agreement encompasses studies, expert statements and consultancy works on the substances concentrated metam, MITC, DMTU and any other metabolite or impurity that both parent substances have in common. Where applicable, Letters of Access or Letters of Co-Ownership, in conjunction with the study reports, are submitted.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Metam Variant 1: Common name: metam-sodium synonyms: metham sodium, metham-Na Variant 2: Common name: metam-potassium synonyms: metham-potassium, metham-K
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	Methyldithiocarbamic acid Variant 1 : metam-sodium : sodium methyldithiocarbamate Variant 2 : metam-potassium : potassium methyldithiocarbamate
CA	*N*-Methylcarbamodithioic acid Variant 1 : metam-sodium : sodium *N*-methylcarbamodithioate Variant 2 : metam-potassium : potassium *N*-methylcarbamodithioate
1.3.3 Producer's development code number	Not applicable.
1.3.4 CAS, EEC and CIPAC numbers	
CAS	144-54-7 Variant 1 : metam-sodium : 137-42-8 Variant 2 : metam-potassium : 137-41-7
EEC	205-632-2 Variant 1 : metam-sodium : 205-293-0 Variant 2 : metam-potassium : 205-292-5
CIPAC	20 Variant 1 : metam-sodium : 20.011 Variant 2 : metam-potassium : 20.019
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_2H_5NS_2$ Variant 1 : metam-sodium : $C_2H_4NNaS_2$ Variant 2 : metam-potassium : $C_2H_4KNS_2$
Structural formula	 Variant 1 : metam-sodium :

	 Variant 2 : metam-potassium : 
Molecular mass	107.2 g/mol Variant 1 : metam-sodium : 129.19 g/mol Variant 2 : metam-potassium : 145.3 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	**CONFIDENTIAL information -** data provided separately **(Document J) (please refer to respective Vol. 4).**
1.3.7 Specification of purity of the active substance in g/kg	**Minimum purity (Annex I - according to COMMISSION IMPLEMENTING REGULATION (EU) No 359/2012 of 25 April 2012):** <u>Technical concentrate (TK):</u> **metam-sodium TK**: min. 400 g/kg – max. 442 g/kg **metam-potassium TK:** min. 520 g/kg – max. 560 g/kg <u>Dry weight basis (calculated):</u> **metam-sodium:** min. 965 g/kg **metam-potassium:** min. 990 g/kg <u>*Note:*</u> the min. purity of 965 g/kg was not the min. purity of metam sodium produced by [redacted] (reference source) which was set to min. 983 g/kg. Min. purity and max. content of the relevant impurities MITC and DMTU were based on the [redacted] source.
	Taminco BV (AIR 5): <u>Technical concentrate (TK):</u> **metam-sodium TK**: min. 400 g/kg – max. 442 g/kg **metam-potassium TK:** min. 520 g/kg – max. 560 g/kg <u>Dry weight basis (calculated – modified equation to derive TC from TK values, refer to Vol. 4):</u> **metam-sodium:** min. 986 g/kg **metam-potassium:** min. 987 g/kg
	Lainco SA (AIR 5): <u>Technical concentrate (TK):</u> **metam-sodium TK**: min. 398 g/kg – max. 439 g/kg **metam-potassium TK:** min. 464 g/kg – max. 470 g/kg <u>Dry weight basis (calculated):</u> **metam-sodium:** min. 982 g/kg (978 g/kg calculated - modified equation to derive TC from TK values, refer to Vol. 4) **metam-potassium:** min. 993 g/kg (992 g/kg calculated - modified equation to derive

		TC from TK values, refer to Vol. 4) **Final specification at renewal is currently pending (please refer to Vol. 4 – Lainco).**
	Final specification at renewal is currently pending (please refer to Vol. 4 – Taminco).	
1.3.8 Identity and content of additives (such as stabilisers) and impurities		
1.3.8.1 Additives	***CONFIDENTIAL information - data provided separately (please refer to respective Vol. 4)***	
1.3.8.2 Significant impurities	***CONFIDENTIAL information - data provided separately (please refer to respective Vol. 4)***	
1.3.8.3 Relevant impurities	**Annex I - according to COMMISSION IMPLEMENTING REGULATION (EU) No 359/2012 of 25 April 2012:** **Methylisothiocyanate (MITC):** max. 12 g/kg on dry weight basis (metam-sodium) max. 0.42 g/kg on dry weight basis (metam-potassium) ***N,N'*-dimethylthiourea (DMTU):** max. 23 g/kg on dry weight basis (metam-sodium) max. 6 g/kg on dry weight basis (metam-potassium)	
	Taminco BV (AIR 5): <u>Dry weight basis (calculated, modified equation to derive TC from TK values, refer to Vol. 4):</u> **Methylisothiocyanate (MITC):** max. 1.2 g/kg **(metam-sodium)** max. 0.5 g/kg **(metam-potassium)** ***N,N'*-dimethylthiourea (DMTU)** max. 13.5 g/kg **(metam-sodium)** max. 6 g/kg on **(metam-potassium)** **Final specification at renewal is currently pending (please refer to Vol. 4 – Taminco).**	**Lainco SA (AIR 5):** <u>Dry weight basis (calculated):</u> **Methylisothiocyanate (MITC):** max. 0.2 g/kg **(metam-sodium)** max. 0.2 g/kg **(metam-potassium)** ***N,N'*-dimethylthiourea (DMTU):** max. 18.7 g/kg **(metam-sodium)** (18.6 g/kg calculated - modified equation to derive TC from TK values, refer to Vol. 4) max. 6.4 g/kg on **(metam-potassium)** **Final specification at renewal is currently pending (please refer to Vol. 4 – Lainco).**
1.3.9 Analytical profile of batches	**CONFIDENTIAL information -** data provided separately **(please refer to respective Vol. 4)**	

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	**Applicant 1- Taminco BV** Company: Taminco BV, a subsidiary of the Eastman Chemical Company Address: Pantserschipstraat 207, B-9000 Gent, Belgium Contact: [redacted] Telephone: [redacted] Fax: not applicable Email: [redacted] **Applicant 2 – Lainco S.A.** Name: Lainco,S.A. Address: Poligono Can Jardi, Avda. Bizet, 8-12, 08191 Rubi, Barcelona, Spain Contact: [redacted] Telephone number: [redacted] Fax number: [redacted] E-mail : [redacted]
1.4.2 Producer of the plant protection product	**Applicant 1- Taminco BV** Company: Taminco BV, a subsidiary of the Eastman Chemical Company Address: Pantserschipstraat 207, B-9000 Gent, Belgium Contact: [redacted] Telephone: [redacted] Fax: not applicable Email: [redacted] **Applicant 2 – Lainco S.A.** Name: Lainco,S.A. Address: Poligono Can Jardi, Avda. Bizet, 8-12, 08191 Rubi, Barcelona, Spain Contact: [redacted] Telephone number: [redacted] Fax number: [redacted] E-mail : [redacted] **Production sites are confidential information provided in the respective Vol. 4.**

1.4.3	**Trade name or proposed trade name and producer's development code number of the plant protection product**	**Applicant 1 – Taminco BV:** Manufacturer's code number: 51053 Proposed trade names: Metam Na 510 g/L: Solasan, Terrasan, Monam, Nemasol, Nemasol 510, Nemasol 510 SL, Sodam 51SL, Scorcher 51 SL, VAPAM FORTE 51SL, Metham Na 51, Tamisol 510, Divapan 51, Fumathane 510, Vapo-solo 510, Metam sódio Quimagro Metam Na 470g/L: Geosaf 39, Geort 50, Vapam Metam K 690 g/L: Tamifum, Tamifum Forte, Tamifume 690 SL, Sodam K 69SL **Applicant 2 – Lainco S.A.:** Manufacturer's code number: Not applicable Trade name: Metam Sodium 51% SL
1.4.4	**Detailed quantitative and qualitative information on the composition of the plant protection product**	

1.4.4.1 Composition of the plant protection product

Applicant 1 – Taminco BV:

Pure active substance

Content of pure active substance:	**Metam-sodium 510 g/L**	**Metam-sodium* (42.1% w/w)**
limits:	485 – 535 g/L	40.1 – 44.2% w/w
Content of pure active substance:	**Metam-potassium 690 g/L**	**Metam-potassium* (54% w/w)**
limits:	665 – 715 g/L	52.0 – 55.9% w/w

* Based on the density of the formulations: 1.21 g/mL (metam-sodium), 1.278 g/mL (metam-potassium)
Note: The preparations are identical to the technical grade active ingredients.
Content of technical active substance: 517.24 g/L (42.75 % w/w).

Applicant 2 – Lainco S.A.:

Content of pure active substance:	**Metam-sodium 510 g/L**	**Metam-sodium* (41.84 % w/w)**
limits:	485 – 535 g/L	39.8 – 43.9 % w/w

Pure active substance
* Based on the density of the formulation: 1.219 g/mL.

1.4.4.2 Information on the active substances

Type	**Name/Code Number**
ISO common name:	metam-potassium
CAS No.:	137-41-7
EC No.:	205-292-5
CIPAC No.:	20.019
Salt, ester anion or cation present:	yes

Type	**Name/Code Number**
ISO common name:	metam-sodium
CAS No.:	137-42-8
EC No.:	205-293-0
CIPAC No.:	20.011
Salt, ester anion or cation present:	yes

1.4.4.3 Information on safeners, synergists and co-formulants

CONFIDENTIAL information - data provided separately **(please refer to respective Vol. 4)**

1.4.5	**Type and code of the plant protection product**	**Applicant 1 – Taminco BV :** **Type:** Soluble concentrate [**Code:** SL] **Applicant 2 – Lainco S.A. :** **Type:** Soluble concentrate [**Code:** SL]
1.4.6	**Function**	Soil fumigant: nematicide, fungicide, herbicide, insecticide
1.4.7	**Field of use envisaged**	**Applicant 1 – Taminco BV:** Metam Na 510 SL is a soluble concentrate (SL) containing 510 g g/L of the active substance metam-sodium and is used against a broad spectrum of soil fungi, cyst nematodes, root-knot and stem nematodes and weeds on many **crops in agriculture and horticulture** (field and protected crops). For the renewal of approval of metam only the indoor application via drip irrigation in permanent greenhouses will be defended, with coverage of the treated area's using Totally Impermeable Film (TIF) for 6 weeks after application. **Applicant 2 – Lainco SA:** Metam Sodium 51% SL is a soil fumigant to be used in pre-planting pre-sowing of **potato, onion and carrot** against *Meloidogyne sp.* (Root-knot nematodes) and Oxalis in outdoor condition and in pre-planting pre-sowing of **pepper** against Nematodes (Root-knot nematodes, needle nematodes, stubby root nematodes, cyst nematodes (*Meloidogyne sp., Pratylenchus sp., Trichodoridae, Ditylenchus sp., Longidorus sp., Heterodera sp*.) and others) in protected condition.
1.4.8	**Effects on harmful organisms**	The active substance metam releases the active metabolite methyl isothiocyanate (MITC). MITC acts as a non-systemic protective fumigant and controls a broad spectrum of soil active plant pathogenic fungi, nematodes; soil insects, emerging weeds and weed seeds. MITC reacts with, and inactivates, the sulfhydryl groups of amino acids and enzymes of living cells. This results in a disruption of lipid metabolism, respiration and the production of ATP. The pesticidal activity is based upon a multi-site contact activity.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

TAMINCO:

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Lettuce	UK, BE, NL, HU, IE, EL, PT, ES, MT, CY, IT, RO, BG, PL, FR	Metam Na 510 SL (Metam K 690 SL)*	G	Nematodes Soil fungi Weeds Insects	SL	510 g/L, expressed in metam-sodium (690 g/L, expressed in metam-potassium)	drip irrigation	6 weeks before planting	1 Application every 3 years	-	0.26 - 1.02 (0.29 – 1.15)*	15000-60000	153 (172)*	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5 - 2.0 % v/v With TIF (6 weeks) Permanent structures
Ornamentals	UK, BE, NL, HU, IE, EL, PT, ES, MT, CY, IT, RO, BG, PL, FR	Metam Na 510 SL (Metam K 690 SL)*	G	Nematodes Soil fungi Weeds Insects	SL	510 g/L, expressed in metam-sodium (690 g/L, expressed in metam-potassium)	drip irrigation	6 weeks before planting	1 Application every 3 years	-	0.26 - 1.02 (0.29 – 1.15)*	15000-60000	153 (172)*	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5 - 2.0 % v/v With TIF (6 weeks) Permanent structures
Baby leaf	UK, BE, NL, HU, IE, EL, PT, ES, MT, CY, IT, RO, BG, PL, FR	Metam Na 510 SL (Metam K 690 SL)*	G	Nematodes Soil fungi Weeds Insects	SL	510 g/L, expressed in metam-sodium (690 g/L, expressed in metam-potassium)	drip irrigation	6 weeks before planting	1 Application every 3 years	-	0.26 -1.02 (0.29 – 1.15)*	7500/30000-10000/40000	77 – 102 (86-115)*	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5 - 2.0 % v/v With TIF (6 weeks) Permanent structures

* Application rate expressed for the equivalent potassium salt product ‘Metam K 690 SL’

LAINCO:

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Potato Carrot Onion	BG, CY, EL, ES, HR, IT, PT	Metam Sodium 51% SL	F	Meloidogyne sp. (Root-knot nematodes) and Oxalis	SL	510 g/L	Soil injection (15-20 cm depth) in combination with Total Impermeable Foil (TIF).	Period: spring to winter. Pre-plant or pre-sowing.	1 application every third year on the same field	n/a	n/a no dilution is made	n/a	153 kg metam-sodium /ha = 127 kg metam/ha	n/a	Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. Soil temperature must be 10-25ºC, soft and slightly moist (60-80% field capacity). Soil should be worked and irrigated 7 to 10 days before application. The product is to be applied undiluted. Metam-sodium is injected in the field at 15 cm depth and immediately homogenised within the soil by spading or roto-tilling in a depth of 25 cm; this allow a good diffusion of the metam-sodium in the whole mass of soil, thus enabling MITC to reach all infested sites. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked in order to allow aeration of any remaining MITC residues and to prepare the soil for sowing or planting. A cress germination test should always be performed prior to sowing or planting in order to confirm the absence

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
															of any remaining MITC in soil that may cause phytotoxic effects.
Pepper	BE, IE, RO, BG, CY, EL, ES, FR, HR, HU, IT, PT, PL	Metam Sodium 51% SL	G ([2])	Nematodes (1) (all crops) CYPSS	SL	510 g/L	Drip irrigation in combination with the use of Total Impermeable Foil (TIF).	Period : spring to winter. Pre-plant or pre-sowing.	1 application every third year	n/a	n/a no dilution is made	n/a	306 kg metam-sodium /ha = 254 kg metam /ha	n/a	Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. Soil temperature must be 10-25ºC, soft and slightly moist (60-80% field capacity). Soil should be worked and irrigated 7 to 10 days before application. The product must be injected into the drip irrigation system undiluted. Metam-sodium is directly injected in the drip-irrigation system by mean of a dosing pump or a venturi set, thus applied as a dilution. Application occurs under plastic mulch to keep the active fumes longer in contact with the organisms to be controlled. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked in order to allow aeration of any remaining MITC residues and to prepare the soil for sowing or planting.

[2] The applicant Lainco clarified that the product is intended to be used in permanent glasshouses as well as walk-in tunnels.

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
															A cress germination test should always be performed prior to sowing or planting in order to confirm the absence of any remaining MITC in soil that may cause phytotoxic effects.

(1) Nematodes : Root-knot nematodes, needle nematodes, stubby root nematodes, cyst nematodes (*Meloidogyne* sp., *Pratylenchus* sp., Trichodoridae, *Ditylenchus* sp., *Longidorus* sp., *Heterodera* sp.) and others.

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Please refer to GAP tables under section 1.5.1.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not applicable.

1.5.4 Overview on authorisations in EU Member States

The currently authorized uses of metam formulations were provided within the document D2 of the renewal dossier of each applicant. The information is presented here below:

Applicant 1 – Taminco BV:

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
BE	Solasan	Metam sodium 510 g/L	Potato seedling cultivation (open air), potato (open air), sugar beet (open air), fodder beet (open air), cherry trees (open air), plum trees (open air), currants (white, red, black) (open air and under protection), blue and red blueberries (open air and under protection), cranberries (open air and under protection), gooseberries and hybrids (open air and under protection), grapes (table grapes) (open air and under protection), grapes (wine production) (open air), raspberry shrubs (open air and under protection), blackberry shrubs (open air and under protection), strawberries (production field) (open air), strawberries (selection and multiplication field) (open air), apple trees (open air), pear trees (open air), carrots (open air), celeriac (open air), black radish and rettich (open air), parsnip (open air), radish (open air and under protection), salsify (open air), turnip (stubble turnip, white turnip) (open air), tomato (under protection), bell pepper and chilli pepper (under protection), aubergine/eggplant and pepino (under protection), cucumber (under protection), gherkin (under protecton), zucchini/patisson (under protection), melon (under protection), pumpkin (under protection), lamb's lettuce (open air and under protection), lettuce varieties (open air and under protection), endive, radicchio rosso and green chicory (open air and under protection), rucola (open air and under protection), spinach (open air and under protection), chicory root cultivation (open air), chard (Swiss chard) (open air and under protection), parsley (consumption leaves) (open air and under protection), chives (consumption leaves) (open air and under protection), chervil (consumption leaves) (open air and under protection), parsley root and chervil root (consumption roots) open air), dill (consumption leaves)	Taminco BV	6412P/B

[3] For new products not yet authorised this field is not applicable.

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
			(open air and under protection), angelica root (consumption leaves) (open air and under protection), cut celery (consumption leaves) (open air and under protection), caraway (consumption leaves) (open air and under protection), coriander (consumption leaves) (open air and under protection), lovage and maggi plant (consumption leaves) (open air and under protection), cicely (consumption leaves) (open air and under protection), tarragon (consumption leaves) (open air and under protection), hyssop (consumption leaves) (open air and under protection), lemon balm (consumption leaves) (open air and under protection), mint (consumption leaves) (open air and under protection), basil (consumption leaves and edible flowers (consumption flowers) (open air and under protection), marjorum/oreganum (consumption leaves) (open air and under protection), rosemary (consumption leaves) (open air and under protection), sage (consumption leaves) (open air and under protection), savoury (consumption leaves) (open air and under protection),thyme (consumption leaves) (open air and under protection), bay laurel (consumption leaves) (open air and under protection), purslane (consumption leaves) (open air and under protection), sorrel (consumption leaves) (open air and under protection), valerian (consumption roots) (open air), borage (consumption leaves) (open air and under protection), salicornia (consumption leaves and/or stems) (open air and under protection), ornamental plants (not destined for consumption) (open air and under protection), potting soil		
BE	Terrasan	Metam sodium 510 g/L	Potato seedling cultivation (open air), potato (open air), sugar beet (open air), fodder beet (open air), cherry trees (open air), plum trees (open air), currants (white, red, black) (open air and under protection), blue and red blueberries (open air and under protection), cranberries (open air and under protection), gooseberries and hybrids (open air and under protection), grapes (table grapes) (open air and under protection), grapes (wine production) (open air), raspberry shrubs (open air and under protection), blackberry shrubs (open air and under protection), strawberries (production field) (open air), strawberries (selection and multiplication field) (open air), apple trees	Taminco BV	7762P/B

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
			(open air), pear trees (open air), carrots (open air), celeriac (open air), black radish and rettich (open air), parsnip (open air), radish (open air and under protection), salsify (open air), turnip (stubble turnip, white turnip) (open air), tomato (under protection), bell pepper and chilli pepper (under protection), aubergine/eggplant and pepino (under protection), cucumber (under protection), gherkin (under protecton), zucchini/patisson (under protection), melon (under protection), pumpkin (under protection), lamb's lettuce (open air and under protection), lettuce varieties (open air and under protection), endive, radicchio rosso and green chicory (open air and under protection), rucola (open air and under protection), spinach (open air and under protection), chicory root cultivation (open air), chard (Swiss chard) (open air and under protection), parsley (consumption leaves) (open air and under protection), chives (consumption leaves) (open air and under protection), chervil (consumption leaves) (open air and under protection), parsley root and chervil root (consumption roots) open air), dill (consumption leaves) (open air and under protection), angelica root (consumption leaves) (open air and under protection), cut celery (consumption leaves) (open air and under protection), caraway (consumption leaves) (open air and under protection), coriander (consumption leaves) (open air and under protection), lovage and maggi plant (consumption leaves) (open air and under protection), cicely (consumption leaves) (open air and under protection), tarragon (consumption leaves) (open air and under protection), hyssop (consumption leaves) (open air and under protection), lemon balm (consumption leaves) (open air and under protection), mint (consumption leaves) (open air and under protection), basil (consumption leaves and edible flowers (consumption flowers) (open air and under protection), marjorum/oreganum (consumption leaves) (open air and under protection), rosemary (consumption leaves) (open air and under protection), sage (consumption leaves) (open air and under protection), savoury (consumption leaves) (open air and under protection),thyme (consumption leaves) (open air and under protection), bay laurel (consumption		

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
			leaves) (open air and under protection), purslane (consumption leaves) (open air and under protection), sorrel (consumption leaves) (open air and under protection), valerian (consumption roots) (open air), borage (consumption leaves) (open air and under protection), salicornia (consumption leaves and/or stems) (open air and under protection), ornamental plants (not destined for consumption) (open air and under protection), potting soil		
BE	Tamifume 690 SL	Metam potassium 690 g/L	Potato seedling cultivation (open air), potato (open air), sugar beet (open air), fodder beet (open air), cherry trees (open air), plum trees (open air), currants (white, red, black) (open air and under protection), blue and red blueberries (open air and under protection), cranberries (open air and under protection), gooseberries and hybrids (open air and under protection), grapes (table grapes) (open air and under protection), grapes (wine production) (open air), raspberry shrubs (open air and under protection), blackberry shrubs (open air and under protection), strawberries (production field) (open air), strawberries (selection and multiplication field) (open air), apple trees (open air), pear trees (open air), carrots (open air), celeriac (open air), black radish and rettich (open air), parsnip (open air), radish (open air and under protection), salsify (open air), turnip (stubble turnip, white turnip) (open air), tomato (under protection), bell pepper and chilli pepper (under protection), aubergine/eggplant and pepino (under protection), cucumber (under protection), gherkin (under protecton), zucchini/patisson (under protection), melon (under protection), pumpkin (under protection), lamb's lettuce (open air and under protection), lettuce varieties (open air and under protection), endive, radicchio rosso and green chicory (open air and under protection), rucola (open air and under protection), spinach (open air and under protection), chicory root cultivation (open air), chard (Swiss chard) (open air and under protection), parsley (consumption leaves) (open air and under protection), chives (consumption leaves) (open air and under protection), chervil (consumption leaves) (open air and under protection), parsley root and chervil root (consumption roots) open air), dill (consumption leaves) (open air and under protection), angelica	Taminco BV	9517P/B

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
			root (consumption leaves) (open air and under protection), cut celery (consumption leaves) (open air and under protection), caraway (consumption leaves) (open air and under protection), coriander (consumption leaves) (open air and under protection), lovage and maggi plant (consumption leaves) (open air and under protection), cicely (consumption leaves) (open air and under protection), tarragon (consumption leaves) (open air and under protection), hyssop (consumption leaves) (open air and under protection), lemon balm (consumption leaves) (open air and under protection), mint (consumption leaves) (open air and under protection), basil (consumption leaves and edible flowers (consumption flowers) (open air and under protection), marjorum/oreganum (consumption leaves) (open air and under protection), rosemary (consumption leaves) (open air and under protection), sage (consumption leaves) (open air and under protection), savoury (consumption leaves) (open air and under protection),thyme (consumption leaves) (open air and under protection), bay laurel (consumption leaves) (open air and under protection), purslane (consumption leaves) (open air and under protection), sorrel (consumption leaves) (open air and under protection), valerian (consumption roots) (open air), borage (consumption leaves) (open air and under protection), salicornia (consumption leaves and/or stems) (open air and under protection), ornamental plants (not destined for consumption) (open air and under protection), potting soil		
NL	Monam	Metam sodium 510 g/L	As a soil fumigant to control nematodes in favor of the outdoor cultivation of: potato As a soil fumigant to control nematodes and fungi in favor of the outdoor cultivation of: strawberry, bulbs and flower bulbs, nurseries, horticultural crops.	Certis Europe B.V.	6443
NL	Nemasol*	Metam sodium 510 g/L	As a soil fumigant to control nematodes in favor of the outdoor cultivation of: potato As a soil fumigant to control nematodes and fungi in favor of the outdoor cultivation of: strawberry, bulbs and flower bulbs, nurseries, horticultural crops.	Taminco BV	9635

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
HU	Nemasol 510	Metam sodium 510 g/L	Vegetables (green peppers, tomatoes, cucumbers, carrots, celery, parsley root), Glasshouse Ornamental Plants/ weeds, root knot nematodes, soil-dwelling pests, soil-dwelling pathogens Disinfection of soil mixtures for seedlings of vegetables and ornamental plants/weeds, root knot nematodes, soil dwelling pests	Eastman Chemical BV	6300/13791-2/2019
PL	Nemasol 510 SL	Metam sodium 510 g/L	Outdoor: Strawberry Indoor: Strawberry	Taminco BV	R-52/2013d
BU	Nemasol 510*	Metam sodium 510 g/L	-	Taminco BV	0363
CY	Nemasol 51 SL	Metam sodium 510 g/L	-	Taminco BV	2404
EL	Nemasol 51 SL*	Metam sodium 510 g/L	-	Taminco BV	6793
EL	Sodam 51SL*	Metam sodium 510 g/L	-	Taminco BV	6945
EL	Scorcher 51 SL*	Metam sodium 510 g/L	-	Taminco BV	6928
EL	VAPAM FORTE 51SL*	Metam sodium 510 g/L	-	Taminco BV	6883
EL	Sodam K 69SL	Metam potassium 690 g/L	-	Taminco BV	60198
IT	Metham Na 51	Metam sodium 510 g/L	-	Eastman Italia srl	9298
IT	Vapam	Metam sodium 470 g/L	-	Eastman Italia srl	3779
IT	Tamisol 510	Metam sodium 510 g/L	-	Eastman Italia srl	10338
IT	Geosaf 39	Metam sodium 470 g/L	-	Eastman Italia srl	11572
IT	Divapan 51	Metam sodium 510 g/L	-	Eastman Italia srl	12981
IT	Fumathane 510	Metam sodium 510 g/L	-	Eastman Italia srl	0565
IT	Geort 50	Metam sodium 470 g/L	-	Eastman Italia srl	0535

MS	Product name	Active substance(s) and content (g/L or g/kg)	Crop(s)	Applicant or authorisation holder	Authorisation number of authorised product[3]
IT	Tamifum	Metam potassium 510 SL	-	Eastman Italia srl	11355
IT	Tamifum Forte	Metam potassium 690 SL	-	Eastman Italia srl	12750
MT	Nemasol	Metam sodium 510 g/L	-	Taminco BV	2010-03-30 P01
PT	Nemasol*	Metam sodium 510 g/L	Outdoor: Strawberry, ornamentals (bulbs & flowers) Indoor: eggplant, strawberry, tomato, cucumber, pepper and lettuce	Taminco BV	1320
PT	Vapo-solo 510	Metam sodium 510 g/L	Outdoor: Strawberry, ornamentals (bulbs & flowers) Indoor: eggplant, strawberry, tomato, cucumber, pepper and lettuce	Taminco BV	1318
PT	Metam sódio Quimagro	Metam sodium 510 g/L	Outdoor: Strawberry, ornamentals (bulbs & flowers) Indoor: eggplant, strawberry, tomato, cucumber, pepper and lettuce	Taminco BV	1319
RO	Nemasol 510	Metam sodium 510 g/L	Indoor : tomatoes	Taminco BV	

*not marketed by Taminco

Applicant 2 – Lainco S.A.:

Country	Trade name	A.S Content	Reg. Number	Registered uses
Cyprus	FUMETHAM 51	510 g/L metam sodium	3242	Vegetables and Ornamental plants (field or greenhouse), Tobacco seeds in greenhouse.
Cyprus	RAISAN 51	510 g/L metam sodium	2468	Vegetables and Ornamental plants (field or greenhouse), Tobacco seeds in greenhouse.
Greece	RAISAN 51 SL	510 g/L metam sodium	6879	Vegetables & Ornamentals (Field and Green house) and Tobacco seedbeds (Greenhouse)
Greece	FUMASOL 51 SL	510 g/L metam sodium	6950	Vegetables & Ornamentals (Field and Green house) and Tobacco seedbeds (Greenhouse)
Greece	LAISOL 40 SL	400 g/L metam sodium	6679	Vegetables & Ornamentals (Field and Green house) and Tobacco seedbeds (Greenhouse)
Ireland	FUMETHAM	510 g/L metam sodium	N01340	Soil fungi, soil insects and weeds
Italy	METAM	510 g/L metam sodium	3745	Tobacco, vegetables as: Tomato, Potato, Beetroot, Cabbage, Melon, Watermelon, Radicchio, Salad, Eggplant, Pepper, Artichoke, Cucumber, Beans, Soya beans, Peas, Asparagus, Garlic, Onion, Carrot, Strawberry, Celery, Chicory, Lettuce; Ornalmental crops in field: Rose, Carnation, Gladiola, Cyclamen, Hydrangea, Tulips, Violets; Tree crops: Citrus, Lemon, Tangerine; Orange, Grapefruit; Grapevine, Apple, Pear and Peach.
Malta	RAISAN 51	510 g/L metam sodium	2015-08-13 P01	Carrot, potato, onion, lettuce, tomato,strawberry, strawberry-nursery - nematodes, soil borne diseases that cause root rot and damping off, soil borne pests, seedlings,rhizomes & tubers of annual & perennial weeds & Oxalis
Portugal (Lainco Portugal Lda.)	RAISAN	510 g/L metam sodium	1295	Pepper, Eggplant, Cucumber and Courgette (in greenhouse) and lettuce, tomato and strawberry (in greenhouse and field)

Portugal (Lainco Portugal Lda.)	LAISOL	*400 g/L metam sodium*	1296	Field: Lettuce, tomato, strawberry. Greenhouse: Pepper, Eggplant, Cucumber, Courgette, Strawberry, Tomato and Lettuce.
Romania	RAISAN 51	*510 g/L metam sodium*	2778	Against pathogens (*Phytophtora parasitica*, *Fusarium oxysporum*, *Verticillium dahliae*), Against nematodes (*Meloidogyne incognita*) and against weeds.

Level 2

METAM

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

A literature search was carried out for the active substance metam including its variants metam-sodium and metam-potassium, its main metabolite MITC (methyl isothiocyanate) and its relevant impurity DMTU (N,N´-dimethylthiourea) (for both applicants), as specified in Article 8(5) of Regulation (EC) No 1107/2009. The Literature Search Report describes the general search and evaluation process as well as details on search profiles, search histories and summary tables. The search and review itself was in accordance with the EFSA Guidance document as published in EFSA Journal 2011;9(2):2092 (European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092).

The objective of the literature search was the assessment of scientific peer-reviewed open literature dealing with side-effects on health, the environment and non-target species published within the last 10 years. Upon request of the Rapporteur Member State, the literature search was extended to open literature published since 1970. Due to the fact that the initial 10-year literature search was not performed within six months before the dossier submission (December 2019), the search was updated (Top-up search). The report is structured into three parts:
- 10-year search (search date: 26th June 2018): Publication year 2008 until 26th June 2018
- 40-year search (search date: 28th February 2019): Publication year 1970 until 2007
- Top-up search (search date: 2nd July 2019): 27th June 2018 until 2nd July 2019

Literature was searched accessing 13 databases: AGRICOLA, BIOSIS, CABA, HCAPLUS, DDFU, EMBASE, ESBIOBASE, MEDLINE, NTIS, TOXCENTER, Food Science and Technology Abstracts (FSTA), PQSCITECH, and SCISEARCH via the service provider STN-International. The search strategy used was a single concept search. The search was performed for the active substance metam, its variants metam-sodium and metam-potassium, its main metabolite MITC (methyl isothiocyanate), its relevant impurity DMTU (N,N´-dimethylthiourea) and other metabolites, using also chemical names, synonyms, trade names and CAS registry numbers as search terms. The search for DMTU and other metabolites (dimethyl urea; N,N'-dimethyl-2(3-methyl-ureido)-acetamide; N,N'-dimethyl-2-(3-methyl-thioureido)-acetamide)) was limited to the initial 10-year search (2008 – June 2018).

A total of 6459 unique summary records were retrieved for review; of these, 5802 were obviously not relevant (based on screening of summary records, i.e. titles and/or abstracts), because there was no link with the active substance metam, it concerned political/economic aspects, it concerned publications in non-EU language without English abstract or because it concerned efficacy, analytical method development or new ways of synthesis. A further 364 references were considered as not relevant after a rapid assessment of the summary record and taking into the relevance criteria (see below).

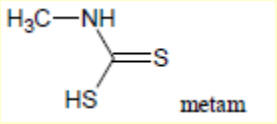
For the remaining 293 references, a more detailed relevance assessment was made by reviewing the full texts according to the relevance criteria. 109 articles remained as possibly relevant and were further considered in the relevant sections of the supplementary dossier.

2.1 IDENTITY

2.1.1 Summary or identity

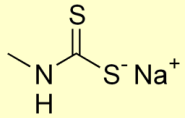
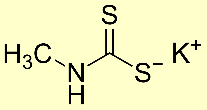
Two individual dossiers (applicant 1 – Taminco BV and applicant 2 – Lainco) have been submitted for this active substance in the frame of the renewal (AIR 5).

Metam (chemical name IUPAC: methyldithiocarbamic acid) was included in Annex I on 01 July 2012 (Reg. (EU) No. 359/2012) for use a nematicide, fungicide, herbicide and insecticide.
Its chemical structure is presented here below:



metam

The active substance metam is available as two variants: metam-sodium (chemical name IUPAC: sodium methyldithiocarbamate) and metam potassium (chemical name IUPAC: potassium methyldithiocarbamate). Both chemical structures are presented here below:

Variant: metam sodium	Variant: metam potassium
	

Both applicants produced both variants of metam.

New 5 batch analyses based on large scale production have been submitted for the purpose of renewal by Taminco BV as well as by the second applicant Lainco SA (please refer to the respective Volume 4: Vol.4 – Taminco and Vol. 4 - Lainco).

During the previous EU review of metam, it was concluded by the peer review in the mammalian toxicology section that the acceptable maximum levels of the relevant impurities were 23 g/kg (dry weight basis) for DMTU and 12 g/kg (dry weight basis) for MITC. Consequently, the min. purity was set to the lower calculated min. purity of the corresponding source [redacted] (i.e. 965 g/kg) not supporting the renewal. The reference source which not triggered the EU agreed levels was at that time considered as covered form a toxicological and ecotoxicological perspective.
An update of the initial reference specification and EU agreed levels appears to be not required from a tox/ecotox perspective. It is however questioned if a revision of the EU agreed levels and an update of the reference specification should not occur and would not be more appropriate in this case even not fully justify from a (eco)tox. perspective (see details in Vol.4 – Taminco and Vol.4 – Lainco).

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

Applicant 1 – Taminco

Purified metam-sodium is a white crystalline powder with a sweet onion-like smell. Its melting point is in the range 86.5 – 90.5 °C and decomposition has been observed at 150°C and at 215°C. It is moderately volatile (vapour pressure = 5.75 x 10^{-2} Pa at 25°C) and readily soluble in water (578.29 g/L at 20°C) and thus, slightly volatile from water (H = 8.34 x 10^{-6} Pa.m³/mol at 20°C). Metam-sodium is also readily soluble in methanol, but in other organic solvents, solubility is relatively low (< 0.26 g/L). It is not a surface-active compound (surface tension of 1 g/L aqueous solution at 21°C: 72.0 mN/m). With a log P_{OW} < -2.9, it is not critical with respect to bioaccumulation. It is not considered as highly flammable or auto-flammable and has no explosive or oxidising properties.

Metam-sodium as manufactured is a clear yellowish liquid with a specific odor. It is not surface-active and not auto-flammable. Flash point was determined to be > 97°C and thus, compliance with FAO specification (Code 20.1Na/13/S/15) (requiring flash point ≥ 66°C) was demonstrated.

Metam-potassium is another variant (salt) of metam. Based on the chemical similarity with metam-sodium, it is expected that vapour pressure, solubility in water and organic solvents, octanol-water partition, dissociation in water, flammability, explosive and oxidising properties of metam-potassium will be comparable to that/those of metam-sodium.

Purified metam-potassium is a pale yellow, crystalline/amorphous powder with a slightly acrid, sulphurous odour. It decomposes (before melting) at a temperature of approximately 150°C. The determined octanol/water partition coefficient (log P_{OW} of –3.44) reconfirms that bioaccumulation of metam-(potassium) in the environment is not to be expected.

Metam-potassium as manufactured is a clear, light yellow liquid (aqueous solution containing 690 g/L metam-potassium) with a pungent, sulphurous odour. The solution is surface-active (surface tension of neat/undiluted preparation and 1g/L aqueous solution: 51.5 and 55.1 mN/m at 20°C, respectively). Furthermore, the aqueous solution is not flammable (flash point > 102°C), not auto-flammable and is not expected to possess explosive or oxidising properties.

MITC *(impurity/main degradation product of metam):*
Purified MITC is a colourless, crystalline solid. It is highly volatile (vapour pressure at 20°C: 1739 Pa) and readily soluble in water (8.94 g/L at 20°C) and thus, moderately volatile from water (H = 14.2 $Pa.m^3/mol$ at 20 ºC). MITC has a log P_{OW} of 1.05 and hence, bioaccumulation of MITC in the environment is not expected

DMTU *(impurity):*
DMTU is a highly volatile substance (vapour pressure of 832.95 Pa at 20°C). It is moderately volatile from water (H = 0.149 $Pa.m^3/mol$ at 20°C), in which it is readily soluble (≥ 581.98 g/L at room temperature). Its low octanol/water partition coefficient (log P_{OW} = - 0.23) indicates that bioaccumulation of DMTU in the environment is not to be expected.

Applicant 2 – Lainco

Pure metam sodium is a white crystalline powder with a sweet onion-like smell. Its melting point is in the range 86.5 – 90.5ºC and decomposition has been observed at 150ºC and at 215ºC. It is moderately volatile (vapour pressure = 5.72 x 10^{-2} Pa at 25ºC) and readily soluble in water (578.29 g/L at 20ºC) and thus, slightly volatile from water (H = 8.34 x 10^{-6} $Pa.m^3.mol^{-1}$ at 20ºC). Metam sodium is also readily soluble in methanol, but in other organic solvents, solubility is relatively low (< 0.26 g/L). It is not a surface active compound (surface tension of 1g/L aqueous solution at 21ºC: 72.0 mN/m). With a log Pow < -2.9, it is not critical with respect to bioaccumulation. It is not considered highly flammable or auto-flammable and has no explosives or oxidising properties.

Metam potassium is another variant (salt) of metam. Based on the chemical similarity with metam sodium it is expected that octanol-water partition, dissociation in water, flammability, explosives and oxidising properties of metam-potassium will be comparable to those of metam sodium.

Purified metam-potassium is a pale yellow, crystalline powder with slightly acrid, sulphurous odour. It decomposes (before melting) at the temperature of approximately 150ºC. The solubility in water could not be determined as instantly dissociates in water due to its ionic structure. The dissociation point based on a pH driven dissociation therefore does not occur as the metam potassium will irreversibly dissociate. The determined octanol/water partition coefficient (log Pow of – 2.61 at pH 7) reconfirms that bioaccumulation of metam potassium in the environment is not to be expected. It is not considered highly flammable or auto-flammable and has no explosives or oxidising properties.
The experimental study to determine Vp for metam potassium has been considered not acceptable.

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance metam (incl. metam sodium and metam potassium)

The properties summarised here below are for both metam sodium and metam potassium variants (produced by applicant 1 – Taminco).

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Metam sodium (97%, purified): White crystalline powder	[redacted] (1997) Report No. FCC 152/963829	Visual inspection (ASTM D1535-89)
	Metam sodium 510 SL (= technical concentrate – TK as manufactured): Yellow clear solution	[redacted] (2001) Report No. W/RL/200101/001	Visual inspection (ASTM D1209-79)
	Metam sodium 510 SL (TK as manufactured): Clear yellowish liquid with a specific odour.	[redacted] (2012) Report No. C67842	Visual
	Metam potassium (98%, purified): Pale yellow amorphous, solid, crystalline powder with a few hard lumps and slightly acrid, sulphurous odour	[redacted] (2009) Report No. J17224/A	Visual inspection
	Metam potassium 690 SL (TK, as manufactured): Clear, light yellow liquid with a pungent odour similar to sulphur	[redacted] (2002a) Report No. 47104	Visual
Melting/freezing point	Metam sodium (99.9%, purified): 86.5 – 90.5 °C	[redacted] (1997) Report No. FCC 152/963829	EEC A1 (Capillary method using metal block)
	Metam potassium (98%, purified): could not be determined due to decomposition (at ~ 150°C, the specimen discoloured and left a yellow/brown residue on the tube walls)	[redacted] (2009) Report No. J17224/A	OECD 102 / EEC A1 (Differential scanning calorimetry + Capillary method using metal block)
Boiling point	Metam sodium (97%, purified): Not applicable (decomposition)	[redacted] (1997) Report No. FCC 152/963829	EEC A16 (thermal analysis method: DTA)
	Metam sodium hydrate (101.84%): The boiling temperature was not determinable as the test item decomposed at temperatures above approximately 155°C (via DSC) without boiling	[redacted] (2019) Report No. JP16TB	EEC A.2, OECD 103 (Siwoloboff method + DSC)
	Metam potassium (98%, purified): could not be determined due to decomposition	[redacted] (2009) Report No. J17224/A	OECD 102 / EEC A1 (Differential scanning calorimetry + Capillary method using metal block)
Relative density	Not a requirement for pure active substance according to Reg. 283/2013 but determined as D_4^{20} = 1.44 (metam sodium, 99.9%, purified)	[redacted] (1997) Report No. FCC 152/963829	Measured (ISO R1183)

Property	Value	Reference	Comment (e.g. measured or estimated)
	Metam sodium 510 SL (TK, as manufactured): D_4^{20} = 1.21 (at 20.0 ± 0.5 °C)	[redacted] (2000c) Report No. 378/038	EEC A3 (pycnometer; ISO 758)
	Not a requirement for pure active substance according to Reg. 283/2013 but determined as D_4^{20} = 1.579 (metam potassium, 98%, purified)	[redacted] (2009) Report No. J17224/A	OECD 109, EEC A3
	Metam potassium (TK) (690 SL) D_4^{20} = 1.278 (at 20.0 ± 0.0 °C)	[redacted] (2002a) Report No. 47104	EEC A3 (pycnometer; ISO 758)
Vapour pressure	Metam sodium (99.9%, purified): 5.75 10^{-2} Pa at 25°C 3.73 x 10^{-2} Pa at 20°C (interpolated from measurements in temperature range 19 to 39°C)	[redacted] (1997) Report No. FCC 152/963829	EEC A.4 (vapour pressure balance method)
	Metam-potassium is an alternative salt variant of the same active metam. The only difference is the counter ion, which is potassium instead of sodium and as such similar physchem properties can be expected. Therefore, no significant difference would be foreseen regarding vapour pressure of both compounds. As a result, metam-potassium is a moderately volatile substance.	-	Statement
Surface tension	Metam sodium (97%): 72.0 mN/m at 21°C (1g/L) (and confirmed at renewal at 101.84%) Metam-sodium (purified) is not considered to be a surface active compound.	[redacted] (1997) Report No. FCC 152/963829 [redacted] (2019) Report No. JP16TB	EEC A5 (OECD harmonised ring method)
	Metam sodium 510 SL (TK): Neat: 63.0 mN/m at 25 °C Metam-sodium (as manufactured) is not considered to be a surface active compound.	[redacted] (2000) Report No. 378/036	EEC A5 (ring method ISO 304)
	Metam potassium 690 SL (TK): Neat: 51.5 mN/m at 20°C + 0.5°C 1 g/L dilution: 55.1 mN/m at 20°C + 0.5°C Metam-potassium (as manufactured) is a surface active compound.	[redacted] (2009) Report No. J17223	EEC A5 (Du-Nüoy Tensiometer – ring method)
Water solubility	Metam sodium (99.9% or 99.2%, purified): 578.29 g/L at 20°C (distilled water; pH increases to 9.2 – 9.3) (99.9 %) 734 g/L at 20°C (pH 9 buffer, pH increases to 10.1) (99.2%) Water solubility is not significantly affected by pH: 600 g/L at 10°C (pH 10.11) 734 g/L at 20°C (pH 10.12) 701 g/L at 30°C (pH 10.37)	[redacted] (1997) Report No. FCC 152/963829 + [redacted] (2002) Report No. UCB 809/013754	EEC A6 (flask method)

Property	Value	Reference	Comment (e.g. measured or estimated)
	Metam potassium: Metam-potassium is an alternative salt variant of the same active metam. The only difference is the counter ion, which is potassium instead of sodium and as such, similar physchem properties can be expected. Therefore, no significant difference would be foreseen regarding water solubility of both compounds. As a result, metam-potassium would also have a high water solubility.	-	statement
Partition coefficient n-octanol/water	Metam sodium (99.9%, purified): log $P_{OW} \leq$ -2.91 at 20°C (pH 6.9)	[redacted] (1997) Report No. FCC 152/963829	EEC A8 (Shake-flask method)
	Metam sodium hydrate (101.84%): At21.2°C: Log Pow = < -2.0 (rounded from -2.32, pH 7, 21.2°C) Log Pow = < -2.0 (rounded from -2.39, pH 9, 21.2°C) Log Pow could not be determined at pH 4 due to the instability of the test item in the aqueous solutions. A value log_{10} P_{OW} of -2.62 (partition coefficient 2.37 x 10^{-3}) was calculated based on fragment constant methodology using KOWWIN v1.68 (September 2010). No significant pH-effect observed	[redacted] (2020) Report No. CQ47NS	EEC A.8 OECD 107 (Shake-flask method)
	Metam potassium (98%, purified): At 19.5 ± 0.5°C log P_{ow} = - 3.42 (pH 5) log P_{ow} = - 3.44 (pH 7) log P_{ow} = - 3.55 (pH 9)	[redacted](2009) Report No. J17224/B	EEC A8 (Shake-flask method)
Henry's law constant	Metam sodium: 8.34 10^{-6} Pa m^3 mol^{-1} at 25°C	Taminco (2007)	Calculation
	Metam potassium: Not determined. Refer to statement under vapour pressure and water solubility between metam-potassium and metam-sodium. As result, Henry's law of metam-potassium refers to the one of metam-sodium.	-	Statement
Flash point	Metam sodium 510 SL (TK, as manufactured): Flash point: > 97°C	[redacted] (2009) Report No. J17220	EEC A9 (Pensky Martens Closed Cup)
	Metam potassium 690 SL (TK, as manufactured): Flash point: > 102°C	[redacted] (2009) Report No. J17223	EEC A9 (Pensky Martens Closed Cup)
Flammability	Not flammable (metam sodium or potassium)	-	The substance is a liquid.
Explosive properties	Metam sodium (97%, purified): Not explosive	[redacted] (1997) Report No. FCC 152/963829	EEC A14

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>Metam sodium 510 SL (TK, as manufactured):
Not explosive (TK; statement + experimental)</td><td>[redacted] (2020)
Report No. KV66CJ</td><td>Differential Scanning Calorimetry (DSC) + theoretical assessment (UN-MTC Screening procedures Annex 6)</td></tr>
<tr><td></td><td>Metam potassium 690 SL (TK, as manufactured)
Not explosive</td><td>[redacted] (2009)
Report No. J17223</td><td>Differential Scanning Calorimetry (DSC) + theoretical assessment</td></tr>
<tr><td>Self-ignition temperature</td><td>Metam sodium 510 SL (TK, as manufactured)
No self-ignition of test substance was observed between room temperature (20°C) and 400 °C.</td><td>[redacted] (2000)
Report No. FCC 378/046</td><td>EEC A15</td></tr>
<tr><td></td><td>Metam potassium 690 SL (TK, as manufactured):
At 342°C, rapid sparking of the samples was observed (stopped after about 1 second).
No distinct ignition occurred.</td><td>[redacted] (2009)
Report No. J17223</td><td>EEC A15</td></tr>
<tr><td>Oxidising properties</td><td>Metam sodium 510 SL (TK, as manufactured):
Not oxidising</td><td>[redacted] (2001)
Report No. UCB 812/013400</td><td>Statement (no oxygen, fluorine or chlorine present)</td></tr>
<tr><td></td><td>Metam potassium 690 SL (TK, as manufactured):
Not oxidising</td><td>[redacted] (2009)
Report No. J17223</td><td>Statement (no oxygen, fluorine or chlorine present)</td></tr>
<tr><td>Granulometry</td><td>No data</td><td>-</td><td>Not relevant. Substance is liquid.</td></tr>
<tr><td>Solubility in organic solvents and identity of relevant degradation products</td><td>Metam sodium (99.9%, purified):
<table><tr><th></th><th>Solubility at 20°C (g/L)</th></tr><tr><td>Heptane</td><td>< 0.2126</td></tr><tr><td>Xylene</td><td>< 0.2611</td></tr><tr><td>1,2-dichloroethane</td><td>< 0.2620</td></tr><tr><td>Ethyl acetate</td><td>< 0.2032</td></tr><tr><td>Acetone</td><td>< 0.2188</td></tr><tr><td>methanol</td><td>33 – 40</td></tr></table></td><td>[redacted] (1997)
Report No. FCC 152/963829</td><td>Method consists of adding measured volumes of solvent to a known mass of test substance until complete dissolution is observed (visual inspection).</td></tr>
<tr><td></td><td>Metam potassium:
Metam-potassium is an alternative salt variant of the same active metam. The only difference is the counter ion, which is potassium instead of sodium</td><td>-</td><td>Statement</td></tr>
</table>

Property	Value	Reference	Comment (e.g. measured or estimated)
	and as such, similar physchem properties can be expected. Therefore, no significant difference would be foreseen regarding solubility in common organic solvents of both compounds. As a result, the results made with metam-sodium could be extrapolated to metam-potassium.		
Dissociation constant	Metam sodium (99.2%, purified): pKa_I = 2.99 and pKa_{II} = 11.06	[redacted] (2002) Report No. UCB 810/013755	OECD 112 (potentiostatic titration)
	Metam potassium: Metam-potassium is an alternative salt variant of the same active metam. The only difference is the counter ion, which is potassium instead of sodium and as such, similar physchem properties can be expected. Therefore, no significant difference would be foreseen regarding dissociation constant of both compounds. As a result, the results made with metam-sodium could be extrapolated to metam-potassium.	-	Statement
Viscosity	Metam sodium 510 SL (TK): A new study was conducted in order The viscosity was measured at shear rates of 171 to 245 s^{-1} at 20°C and 40°C. <u>Dynamic viscosity</u> 4.7 mPa.s at 20°C 2.8 mPa.s at 40°C <u>Kinematic viscosity (calculated)</u> 3.8 mm^2/s at 20°C 2.3 mm^2/s at 40°C Metam Na 510 SL can be regarded as Newtonian fluid	[redacted] (2019) Report No. PL87HX	CIPAC MT 192 OECD 114 OCSPP 830.7100
	Metam potassium 690 SL (TK): <u>Dynamic viscosity</u> 20.0°C: 3.7 cP [mPa.s] at 50 rpm 3.8 cP [mPa.s] at 100 rpm 40.0°C: 2.8 cP [mPa.s] at 50 rpm 2.6 cP [mPa.s] at 100 rpm Metam K 690 SL can be regarded as Newtonian fluid. <u>Kinematic viscosity (calculated)</u> 20.0°C: 2.9 mm^2/s at 50 rpm 3.0 mm^2/s at 100 rpm 40.0°C: 2.2 mm^2/s at 50 rpm 2.3 mm^2/s at 100 rpm	[redacted] (2002b) Report No. 47106	OPPTS 830.7100 (rotational viscometer)
Spectra (UV/VIS, IR,	Metam-sodium (99.9%, purified):	[redacted] (1997)	OECD 101

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>
<table>
<tr><th></th><th>λ_{max} (nm)</th><th>ε (L mol^{-1} cm^{-1})</th></tr>
<tr><td rowspan="4">Neutral (distilled water)</td><td>205.0</td><td>7502.8</td></tr>
<tr><td>248.0</td><td>7942.3</td></tr>
<tr><td>280.0</td><td>9924.0</td></tr>
<tr><td>at λ 290 nm</td><td>> 10</td></tr>
<tr><td rowspan="3">Alkaline (0.1 M NaOH)</td><td>248.0</td><td>8042.5</td></tr>
<tr><td>280.0</td><td>9796.8</td></tr>
<tr><td>at λ 290 nm</td><td>> 10</td></tr>
<tr><td>Acidic (0.1 M HCl)</td><td colspan="2">Metam-sodium hydrolysed too fast to measure accurately</td></tr>
</table>
Supplementary information Metam sodium hydrate (101.84%):
<table>
<tr><th></th><th>λ_{max} (nm)</th><th>ε (L.mol^{-1}.cm^{-1})</th></tr>
<tr><td rowspan="4">Neutral
Purified water
pH 7.8</td><td>210</td><td>9004</td></tr>
<tr><td>249</td><td>10060</td></tr>
<tr><td>281</td><td>12040</td></tr>
<tr><td>290</td><td>7653</td></tr>
<tr><td rowspan="3">Alkaline
(0.1 M NaOH)
pH 13.2</td><td>249</td><td>9680</td></tr>
<tr><td>281</td><td>11110</td></tr>
<tr><td>290</td><td>6981</td></tr>
<tr><td rowspan="4">Acidic
(0.1M HCl)
pH 1.4</td><td>206</td><td>15900</td></tr>
<tr><td>235</td><td>6355</td></tr>
<tr><td>266</td><td>8175</td></tr>
<tr><td>290</td><td>1394</td></tr>
</table>
</td><td>Report No. FCC 152/963829
+
[redacted]
(2019)
Report No. JP16TB</td><td></td></tr>
<tr><td></td><td>Metam potassium (98%, purified):
<table>
<tr><th></th><th>λ_{max} (nm)</th><th>ε (L.mol^{-1}.cm^{-1})</th></tr>
<tr><td rowspan="4">Neutral (pH 6.94)</td><td>206</td><td>8318</td></tr>
<tr><td>248</td><td>7357</td></tr>
<tr><td>282</td><td>8954</td></tr>
<tr><td>at λ 290 nm</td><td>approx. 6400</td></tr>
<tr><td rowspan="4">Alkaline (pH = 10.88)</td><td>195</td><td>14664</td></tr>
<tr><td>248</td><td>7397</td></tr>
<tr><td>282</td><td>8908</td></tr>
<tr><td>at λ 290 nm</td><td>approx. 6500</td></tr>
<tr><td>Acidic (pH = 1.90)</td><td colspan="2">Sample was unstable. (no accurate measurement possible due to rapid hydrolysis of metam potassium)</td></tr>
</table>
</td><td>[redacted]
(2009)
Report No. J17224/A</td><td>OECD 101</td></tr>
</table>

Table 1-2: Table of physicochemical properties of MITC

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless crystalline solid (97.0%)	[redacted](1997) Report No. FCC	Visual inspection

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td></td><td>153/962827</td><td></td></tr>
<tr><td>Melting/freezing point</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Boiling point</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Relative density</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Vapour pressure</td><td>1739 Pa (20°C, 99.4 %)</td><td>[redacted] (2003) Report No. RCC 850435</td><td>OECD 104 EEC A.4 (Gas saturation method)</td></tr>
<tr><td>Surface tension</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Water solubility</td><td>8.94 g/L at 20°C (97.0%, pH 7.5)</td><td>[redacted] (1997) Report No. FCC 153/962827</td><td>EEC A6 (Flask method)</td></tr>
<tr><td>Partition coefficient n-octanol/water</td><td>log P_{OW} at 20°C: 1.05 (pH 7.5, 97.0 %)</td><td>[redacted] (1997) Report No. FCC 153/962827</td><td>EEC A8 (Shake-flask method)</td></tr>
<tr><td>Henry’s law constant</td><td>H = 14.2 Pa m^3 mol^{-1} (20°C)</td><td>Taminco (2007)</td><td>Calculation</td></tr>
<tr><td>Flash point</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Flammability</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Explosive properties</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Self-ignition temperature</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Oxidising properties</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Granulometry</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Solubility in organic solvents and identity of relevant degradation products</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Dissociation constant</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Viscosity</td><td>No data</td><td>-</td><td>-</td></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>97% pure:
<table>
<tr><th></th><th>λ_{max} (nm)</th><th>ε (L mol^{-1} cm^{-1})</th></tr>
<tr><td rowspan="3">Neutral (distilled water)</td><td>235</td><td>635</td></tr>
<tr><td>283</td><td>65</td></tr>
<tr><td>321</td><td>17</td></tr>
<tr><td rowspan="3">Alkaline (0.1 M NaOH)</td><td>276</td><td>46</td></tr>
<tr><td>320</td><td>5</td></tr>
<tr><td>at λ 290 nm</td><td>Not reported, but > 10 based on spectrum</td></tr>
<tr><td rowspan="2">Acidic (0.1 M HCl)</td><td>235</td><td>635</td></tr>
<tr><td>283</td><td>23</td></tr>
</table></td><td>[redacted] (1997) Report No. FCC 153/962827</td><td>OECD 101</td></tr>
</table>

Property	Value			Reference	Comment (e.g. measured or estimated)
		324	6		
		at λ 290 nm	Not reported, but > 10 based on spectrum		

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC 14	No explosion was observed when submitting the test substance to the effect of a flame (thermal sensitivity) or to shock (mechanical sensitivity) or friction (sensitivity to mechanical stimuli). Not explosive	Metam sodium (97%, purified)	[redacted] (1997) Report No. FCC 152/963829
Differential Scanning Calorimetry (DSC) + theoretical assessment (UN-MTC Screening procedures Annex 6)	Based on the chemical structure and DSC result (thermal behaviour) of the test item (heated up to 500°C with no significant exothermic event observed) the result for the explosive properties has been predicted negative. Not explosive	Metam sodium 510 SL (TK, as manufactured):	[redacted] (2020) Report No. KV66CJ
Differential Scanning Calorimetry (DSC) + theoretical assessment	*DSC examination (for thermal explosivity):* heating test specimen from 30° to 400°C did not result in a significant exothermic event. *Theoretical assessment:* Metam-K does not possess chemical groups associated with explosive properties (e.g. – NO_2, -NO_3, -N_3, peroxide, chlorate, perchlorate, nitrate, bromate, chromate) and the rest of the formulation is water, which poses no risk with regard to explosivity. Not explosive	Metam potassium 690 SL (TK, as manufactured)	[redacted] (2009) Report No. J17223

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

The explosive properties of metam-sodium were determined according to EEC Method A14 and under GLP. No explosion was observed when submitting the test substance to the effect of a flame (thermal sensitivity) or to shock (mechanical sensitivity) or friction (sensitivity to mechanical stimuli). Based on the chemical structure and DSC

result of the test item (heated up to 500°C) the result for the explosive properties has been predicted negative using the UN-MTC Screening procedures Annex 6.
The explosive properties of metam-potassium were determined performing differential scanning calorimetry and a theoretical assessment.
DSC examination: heating test specimen from 30° to 400°C did not result in a significant exothermic event.
Theoretical assessment: Metam-K does not possess chemical groups associated with explosive properties (e.g. -NO2, -NO3, -N3, peroxide, chlorate, perchlorate, bromate, chromate) and the rest of the formulation is water, which poses no risk with regard to explosivity.

2.2.1.1.1.2 Comparison with the CLP criteria

The test required for CLP classification regarding explosive properties is the United Nations Recommendations on the Transport of Dangerous Goods (UN RTDG) Manual of Tests and Criteria ST/SG/AC.10/11/ Rev. 5 – Part I (Test series), section 11. However, since no sign of explosion were observed during the test according to EEC-Method A14, and taking into account that metam (incl. metam sodium and metam potassium) does not contain structural elements or functional groups that are consistent with explosivity, tt can be reasonably assumed that metam (incl. metam sodium and metam potassium) does not exhibit explosive properties.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Metam (incl. metam sodium and metam potassium) is not an explosive. Data conclusive but not sufficient for classification.
MITC: No relevant data available.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Metam (incl. metam sodium and metam potassium): not relevant.
Metam sodium and metam potassium are liquids.
MITC: not relevant. MITC is a solid.

2.2.1.1.2.2 Comparison with the CLP criteria

See 2.2.1.1.2.1.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

See 2.2.1.1.2.1.

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Metam (incl. metam sodium and metam potassium): not relevant. Metam sodium and metam potassium are liquids.
MITC: not relevant. MITC is a solid.

2.2.1.1.3.2 Comparison with the CLP criteria

See 2.2.1.1.3.1.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

See 2.2.1.1.3.1.

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Metam (incl. metam sodium and metam potassium): not relevant. Metam sodium and metam potassium are liquids.
MITC: not relevant. MITC is a solid.

2.2.1.1.4.2 Comparison with the CLP criteria

See 2.2.1.1.4.1.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

See 2.2.1.1.4.1.

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EEC A9 (Pensky Martens Closed Cup)	No flash point observed. At a temperature of 51 to 52°C, a change in colour of the test flame was noted. At a temperature of 97°C, sample boiled and extinguished the flame. Flash point: > 97°C	Metam sodium 510 SL (TK, as manufactured)	[redacted] (2009) Report No. J17220
EEC A9 (Pensky Martens Closed Cup)	Flash point: No flash point observed. At a temperature of 102°C, sample boiled and extinguished the flame. Flash point: > 102°C	Metam potassium 690 SL (TK, as manufactured)	[redacted] (2009) Report No. J17223

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

The flash point was determined according to EEC Method A9 and CIPAC Method MT 12.3, employing a Pensky-Martens Closed Cup Tester and Stirrer and an IP 15C thermometer and under GLP. No flash point was observed. At a temperature of 97°C for metam-sodium and 102°C for metam-potassium the sample boiled and extinguished the flame.

2.2.1.1.5.2 Comparison with the CLP criteria

A substance shall be classified as flammable liquid when it has a flash point of not more than 60°C. It is concluded that metam (incl. sodium and –potassium) is not flammable liquid as no flash point was observed and therefore do not meet the criteria for classification as flammable liquid.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Metam (incl. metam sodium and metam potassium) is not a flammable liquid. Data conclusive but not sufficient for classification.
MITC: Hazard class not applicable: The substance is a solid.

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Metam (incl. metam sodium and metam potassium): not relevant. Metam sodium and metam potassium are liquids.
MITC: no data.

2.2.1.1.6.2 Comparison with the CLP criteria

See 2.2.1.1.6.1.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

See 2.2.1.1.6.1.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Metam (incl. metam sodium and metam potassium): data lacking. No study to be conducted because there are no chemical group associated with self-reactive properties present in the molecule of metam (incl. sodium and potassium) (see explosive properties).
MITC: data lacking.

2.2.1.1.7.2 Comparison with the CLP criteria

Metam (incl. metam sodium and metam potassium) contains none of the chemical groups indicated in the screening procedure for this hazard class and associated with explosive or self-reactive properties according to Appendix 6 Sections 3.1 (Table A6.1) and 5.1 (Table A6.2) of the UN MTC.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Metam (incl. metam sodium and metam potassium) has no self-reactive properties. Data conclusive but not sufficient for classification.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Metam (incl. metam sodium and metam potassium): data lacking. The study does not to be conducted because the substance is known to be stable in contact with air at room temperature for prolonged periods of time (days) and hence, the classification procedure does not need to be applied.
MITC: Not relevant. MITC is a solid.

2.2.1.1.8.2 Comparison with the CLP criteria

A “no classification” proposal based on experience in handling is considered sufficient. The overall classification conclusion is that metam (incl. metam sodium and metam potassium) is not classified as pyrophoric liquid. Data conclusive but not sufficient for classification.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Metam (incl. metam sodium and metam potassium) is no classified as pyrophoric liquid. Data conclusive but not sufficient for classification.

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Metam (incl. metam sodium and metam potassium): not relevant. Metam sodium and metam potassium are liquids.
MITC: no data.

2.2.1.1.9.2 Comparison with the CLP criteria

See 2.2.1.1.9.1.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

See 2.2.1.1.9.1.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A15	No self-ignition of test substance was observed between room temperature (20°C) and 400 °C.	Metam sodium 510 SL (TK, as manufactured)	[redacted] (2000) Report No. FCC 378/046
EEC A15	At 342°C, rapid sparking of the samples was observed (stopped after about 1 second). No distinct ignition occurred.	Metam potassium 690 SL (TK, as manufactured)	[redacted] (2009) Report No. J17223
EEC A1 (Capillary method using metal block)	Melting point: 86.5 – 90.5 °C	Metam sodium (99.9%, purified)	[redacted](1997) Report No. FCC 152/963829
OECD 102 / EEC A1 (Differential scanning calorimetry + Capillary method using metal block)	Melting point could not be determined due to decomposition (at ~ 150°C, the specimen discoloured and left a yellow/brown residue on the tube walls)	Metam potassium (98%, purified)	[redacted] (2009) Report No. J17224/A

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

Metam sodium and metam potassium were submitted to the self-ignition test according to EEC-Method A15. The test was performed under GLP. The substance is not self-igniting below 400°C for metam sodium and 342°C for metam potassium.
Melting point was determined according to EEC A1/OECD 102 methods. The test was performed under GLP. Melting point of metam sodium was determined to be 86.5 – 90.5°C and metam potassium decomposed at 150°C before melting.

2.2.1.1.10.2 Comparison with the CLP criteria

According to the CLP regulation, the Test N.4: test method for self-heating substances (UN RTDG Manual of Tests and Criteria ST/SG/AC.10/ 11/Rev. 5 – Part III, section 33.3.1.6) is recommended to determine the self-heating classification under the CLP. Test N.4 was not carried out for metam. metam sodium and metam potassium were only submitted to the self-ignition test according to EEC-Method A15 (metam sodium and metam potassium were found not self-igniting below 400°C and 342°C, respectively). Results from the test method EEC A15 for liquid are not sufficient to conclude on this hazard class. Nevertheless, in general, the phenomenon of self-heating applies only to solids andliquid are in general not classified as self-heating substances. Substances with a low melting point, < 160°C, should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced. The melting point for metam sodium was found to be 86.5 – 90.5°C and the melting point for metam potassium could not be determined because of decomposition at 150°C before melting. In each case, the temperature is below the trigger temperature of 160°C and metam (incl. metam sodium and metam potassium) is considered to not exhibit self-heating properties.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Metam (incl. metam sodium and metam potassium) has no self-heating properties. Data conclusive but insufficient for classification.
MITC: No data.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Metam (incl. metam sodium and metam potassium): data lacking. However, based on experience in manufacture and handling the substance does not emit flammable gases in contact with water. The product is water-based.
MITC: no relevant data available.

2.2.1.1.11.2 Comparison with the CLP criteria

See 2.2.1.1.11.1. Metam (incl. -sodium and -potassium) does not meet the criteria to be classified as releasing flammable gas in contact with water.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Metam (incl. -sodium and -potassium): Not releasing flammable gas in contact with water. Data conclusive but not sufficient for classification.
MITC: No relevant data available.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
/	Not oxidising	Metam sodium 510 SL (TK, as manufactured):	Statement (no oxygen, fluorine or chlorine present)
/	Not oxidising	Metam potassium 690 SL (TK, as manufactured):	Statement (no oxygen, fluorine or chlorine present)

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

The substance does not contain oxygen, fluorine or chlorine. The rest of the formulation is water, which poses no risk with regard to oxidising properties.

2.2.1.1.12.2 Comparison with the CLP criteria

For organic substances the classification procedure for this class does not need to be applied if the substance does not contain oxygen, fluorine or chlorine. Hence the substance has not to be classified as oxidising liquid according CLP Regulation.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Metam (incl. metam sodium and metam potassium) is not an oxidising liquid.
Data conclusive but not sufficient for classification.
MITC: Hazard class not applicable: the substance is a solid.

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

Metam (incl. metam sodium and metam potassium): not relevant. Metam sodium and metam potassium are liquids.
MITC: no data.

2.2.1.1.13.2 Comparison with the CLP criteria

See 2.2.1.1.13.1.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

See 2.2.1.1.13.1.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

Metam (incl. -sodium and -potassium): Hazard class not applicable: The substance is not an organic peroxide according to the CLP definition.
MITC: Hazard class not applicable: The substance is not an organic peroxide according to the CLP definition.

2.2.1.1.14.2 Comparison with the CLP criteria

See 2.2.1.1.14.2.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

See 2.2.1.1.14.2.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

There were no studies conducted in accordance with UN Test C.1 to test corrosivity to metals.
Based on the experience Metam (incl. -sodium and -potassium) is claimed to be corrosive to metals. Since the active substance is classified as skin corrosive 1 (H314) (please refer to section 2.6.2.4 here below for reassessment and conclusion of this hazard class for the renewal of the active substance according to Reg. 1107/2009), the active substance is automatically classified as corrosive to metals (H290).

In the course of the assessment, RMS requested additional evidence to support that metam (incl. metam sodium and metam potassium) should be classified as corrosive to metals than rather a proposal for classification in this hazard class only based on the skin corrosive classification. The applicant therefore submitted a statement (Topic: [redacted] (2015) - Statement concerning the corrosion effect of Solasan (6412P/B), Terrasan (7762P/B) and Tamifume 690 SL (9517P/B) – Taminco BV (2015), including extracts from the metam sodium product stewardship manual).
The following was taken from the extract of the Product Stewardship Manual metam sodium submitted by the applicant in annex of the statement:

* **Material compatibility. Metam-Sodium** is known to be corrosive to certain materials and the product may soften or discolor iron. The following compatibility table indicates materials that are compatible and non-compatible with **Metam-Sodium**:

COMPATIBLE MATERIALS	NON-COMPATIBLE MATERIALS
carbon steel (coated)	aluminum
304 stainless steel	brass
316 stainless steel	Buna-N
high density or cross link polyethylene	copper
	galvanized steel
	low density polyethylene
	PVC[1]
	zinc

Note: Uncoated steel or cast iron will lead to discoloration of **Metam-Sodium** and the possibility of fine solids formation which can be very difficult to remove by filtration. The corrosion rate of mild steel by **Metam-Sodium** is low, and is otherwise acceptable for use with the product.

2.2.1.1.15.2 Comparison with the CLP criteria

According to the CLP Regulation, the UN Test C.1 (described in part III, Section 37.4.1.1 of the UN-MTC) is recommended to determine the "corrosive to metals" classification under the CLP. Test C.1 was not carried out because based on experience, metam (incl. metam sodium and metam potassium) is known to be corrosive to certain metals, including aluminum and carbon steel (most common metals taken by convention for assessing corrosion to metal for the purposes of CLP). In such case and according to the screening procedure and waiving of testing for this hazard class, additional testing is not needed (section 2.16.4 of the CLP guidance).

If the non-compatibility of metam sodium against certain metals observed from the experience is compared with the results presented in table 2.4 of section 2.16.7 of the CLP guidance (examples of classification for substances and mixtures corrosive to metals), metam sodium should be classified as Met. Corr. 1 (H290) since according to Table 2.4, a classification as corrosive to metals is proposed each time one of the tested metals (steel or aluminium) or both are corroded. The same classification is assumed for metam potassium.

Substance	**Steel*** **	**Aluminium**	**CLP Annex I, 2.16 classification**	**Skin corrosive**
Hydrochloric acid (diluted) (UN1789)	Corroded	Corroded	Classified	Negative
Metam sodium	Corroded*	Corroded*	Classified	Positive**

* Based on experience.

** Please refer to section 2.6.2.4 for discussion and conclusion on the skin corrosive cat. 1. Classification.

*** Carbon steel according to the CLP guidance. For metam sodium: galvanized steel according to the extract of the Product Stewardship Manual metam sodium.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Metam (incl. -sodium and -potassium): data lacking but metam is claimed by applicant to be classified – corrosive to metals, Category 1 (H290: may be corrosive to metals) due to experience and to classification as skin corrosive.
RMS: pH of the product is further > 9 (undiluted).

The reference to experience made by the applicant should be clearly supported by a clear rationale and any experimental evidences/observations that corrosion occurs. Otherwise, RMS thinks that the UN C1 test should occur.

In the course of the assessment, the applicant provided additional information (Topic: [redacted] (2015) - Statement concerning the corrosion effect of Solasan (6412P/B), Terrasan (7762P/B) and Tamifume 690 SL (9517P/B) – Taminco BV (2015), including extracts from the metam sodium product stewardship manual):

"*As indicated previously corrosion tests compliant with the criteria stipulated in the CLP Regulation (Reg. 1272/2008) are not available. As the metam-sodium and metam-potassium products are already classified for skin corrosion with H314 and linked GHS05 symbol, separate metal corrosion tests were not conducted. However metam-*

sodium is known to be corrosive to certain materials, including metals. The product stewardship manual for metam-sodium indicates that metam-sodium is not compatible with aluminium, brass, copper, galvanised steel and zinc. Non compatibility with certain metals is also assumed for metam-potassium. For this reason, we believe it is justified to additionally classify the metam-sodium and metam-potassium products with H290 'May be corrosive to metals'."

Some experimental evidences of such incompatibilities observed on site, if any, should however be provided in full support of the statement.
Overall metam should be classified as Met. Corr. 1, H290

MITC: Not classified

2.2.2 Summary of physical and chemical properties of the plant protection product

Applicant 1 - Taminco

The preparation ***Metam-sodium 510 g/L*** *(soluble concentrate, SL)* is identical to the technical active ingredient as manufactured. It is a clear, yellowish solution with a specific odor and has a relative density (D_4^{20}) of 1.21 (at 20°C). Furthermore, metam-sodium 510 g/L is not considered auto-flammable and is not expected to have explosive or oxidising properties. The preparation has a pH around 9.6 and is not surface-active. No significant physical/chemical changes were observed after storage for 14 days at 54°C or for 7 days at 0°C. Moreover, the preparation was shown to remain stable after storage for 2 years at ambient conditions.
The flash point was determined to be > 97°C and thus, compliance with FAO specification (Code 20.1Na/13/S/15) (requiring flash point ≥ 66°C) was demonstrated.

The preparation ***Metam-potassium 690 g/L*** *(soluble concentrate, SL)* is identical to the technical metam-potassium as manufactured. It is a clear yellow liquid of low viscosity with a faint odour and a relative density of 1.278 (at 20°C). The preparation has Newtonian fluid behaviour and is surface-active (surface tension of neat formulation and 1g/L aqueous solution: 51.5 and 55.1 mN/m at 20°C, respectively). A 1% (w/w) aqueous dilution has a pH of 7.92 (at 22°C). Furthermore, the preparation is not flammable (flash point > 102°C), not auto-flammable and no explosive or oxidising properties are to be expected.
Metam-potassium 690 g/L was found to be physically stable to storage at 0 °C for 7 days. The preparation was also shown to remain stable after storage for 2 years at ambient conditions. No persistent foam is formed when the preparation is diluted in water.

Applicant 2 - Lainco

Metam Sodium 51% SL [Metam-sodium 510 g/L SL] is a transparent liquid of orange colour, with a characteristic odour. Based on the constituents of the formulation, it has no explosive properties. It has a self-ignition temperature of above 400°C. It has a pH value around 8.69 – 8.99 in a 1 % aqueous solution. The stability data indicate a shelf life of at least 2 years at ambient temperature. Its technical characteristics are acceptable for a soluble concentrate formulation.

RMS:
- **The notifier Lainco stated that the packaging and closure have been used for many years and tested several times with no observation of incompatibility or deformation. Nevertheless, description of packaging during storage stability studies provided was not discussed and some experimental evidence (determination of weight loss, deformation, etc.) should be provided. This could still be addressed later at zonal level.**
- **Surface tension should also be determined on the neat formulation. This could however be still addressed later at zonal level.**

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Typical pests which can appear in many outdoor grown crops and which can be controlled or at least reduced to an acceptable level by metam are phytopathogenic soil fungi and nematodes, and grass- and broadleaved weeds. Efficacy is well-known against representative pest species of these pathogen classes for the representative uses and was evaluated for several authorised products.

2.3.2 Summary of information on the development of resistance

Resistance is unlikely to occur since the product acts on various enzymes involved in the cellular respiration process. Indeed, the appearance of a resistant strain of organisms would mean that such an organism is capable to modify and adapt at the same time numerous of its enzymes, which is known to be practically impossible.

RMS: No record of resistance to metam is reported on the website https://www.pesticideresistance.org

2.3.3 Summary of adverse effects on treated crops

The waiting period between treatment and planting or sowing the new crop depends on the temperature, the moisture, the structure and texture of the soil. For soils with mean moisture content the following indicative waiting periods were proposed by the applicant for the DAR revised in June 2008:

Soil temperature at 10 cm depth	Waiting period between application and planting/sowing (days)
15 - 18 °C	13 - 15
12 - 15 °C	16 - 20
8 - 12 °C	21 - 35
6 - 8 °C	36 - 45

To determine if a new crop can be planted or sown without risk of phytotoxicity, a cress test is recommended.

2.3.4 Summary of observations on other undesirable or unintended side-effects

The plants are grown in treated soils only after complete disappearance of the active ingredient Metam sodium and its active metabolite MITC. A cress germination test should always be performed prior to sowing or planting in order to confirm the absence of any remaining MITC in soil that may cause phytotoxic effects.
Application is made to bare soil, the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is immediately covered by a total impermeable foil. This mode of application excludes any potential for off-field exposure of non-target plants.
Concerning the effects on beneficial and other non-target organisms, see ecotoxicological evaluation.

2.4 FURTHER INFORMATION

Both applicants provided MSDSs for metam sodium and metam potassium. The following information is based on these MSDSs. Recommended methods and precautions concerning handling, storage, transport and firefighting measures between the active substances (metam sodium and metam potassium) and the corresponding formulations are identical since the active substance (incl. metam sodium and metam potassium) is produced as a TK, identical or almost identical to the corresponding end formulation.

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Taminco BV

Advice on safe handling:	Avoid inhalation of vapour or mist. Do not get on skin or clothing. Do not get in eyes. Avoid contact with skin, eyes and clothing.

	Do not swallow. Ensure adequate ventilation. Wash thoroughly after handling.
Advice on protection against fire and explosion:	Keep product and empty container away from heat and sources of ignition. Take precautionary measures against static discharges.
Hygiene measures:	Handle in accordance with good industrial hygiene and safety practice.

Lainco SA

- Handle the product container with care, avoiding, during its transport, crushes with heavier products and not letting it fall down.
- Before applying the product be sure that the equipment you will use is the proper one and it is in perfect state. Follow the instructions for the product preparation indicated on the label of the container.
- Mark the treated zones, forbidding people not wearing appropriate protective equipment to enter.
- Have in hand the appropriate devices to wash eyes or skin in case of an accident. Avoid the skin contact with the product and inhalation of vapours/aerosols. Work always in favour of the wind.
- Do not eat, drink, or smoke during the manipulation of the product. Take off the stained or soaked clothing with product immediately and wash with water and soap before using it again. Do not put dirty rags, stained with the product in the pockets.
- Avoid the contact with the product.
- Precautions against fire and explosion hazards: work in places with appropriate ventilation and far from possible ignition sources. Extinguish any flame and avoid heat and static electric sources. Considering that the product can be electrostatically charged always ground containers when transferring. No smoking.

Precautions concerning storage:

Taminco BV
Requirements for storage areas and containers: Keep tightly closed.

Lainco SA

- Store at room temperature.
- At low temperatures it may crystallise. Heat slightly and dissolve before using.
- Store in original packages perfectly closed, in a cold, dry and ventilated place. Use polyethylene packages, sealed. In contact with soil, water, oxygen and acids may give off toxic gases. Possible decomposition products are: Methyl Isothiocyanate (MITC) (main product), carbon sulphide, sulphydric acid, methylamine, carbonyl sulphide, nitrogen disulphide, N'-dimethylthiouram thiourea and sulphur. Incompatible products: In aqueous solution corrodes aluminium, copper, zinc and bronze. Packing material: Keep only in the original container. Use polyethylene packages, sealed.

Precautions concerning transport:

Taminco BV

ADN

UN number:		UN 3267
UN proper shipping name:	Metam-sodium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (metam sodium)
	Metam-potassium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-Potassium 54% in water)
Transport hazard class(es):		8
Packing group	Packing group:	II
	Classification Code:	C7
	Hazard Identification Number:	80
	Labels:	8
Environmentally hazardous:		yes

ADR

UN number:		UN 3267
UN proper shipping name:	Metam-sodium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (metam sodium)
	Metam-potassium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-Potassium 54% in water)
Transport hazard class(es):		8
Packing group:	Packing group:	II
	Classification Code:	C7
	Hazard Identification Number:	80
	Labels:	8
	Tunnel restriction code:	(E)
Environmentally hazardous:		yes

RID

UN number:		UN 3267
UN proper shipping name:	Metam-sodium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (metam sodium)
	Metam-potassium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (metam potassium)
Transport hazard class(es):		8
Packing group:	Packing group:	II
	Classification Code:	C7
	Hazard Identification Number:	80
	Labels:	8
Environmentally hazardous:		yes

IMDG

UN number:		UN 3267
UN proper shipping name:	Metam-sodium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (metam sodium)
	Metam-potassium:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-Potassium 54% in water, metam potassium)
Transport hazard class(es):		8
Packing group:	Packing group:	II
	Labels:	8
	EmS Code:	F-A, S-B
Marine pollutant:		yes

IATA

UN number:		UN 3267
UN proper shipping name:	Metam-sodium:	Corrosive liquid, basic, organic, n.o.s. (metam sodium)
	Metam-potassium:	Corrosive liquid, basic, organic, n.o.s. (Metam-Potassium 54% in water)
Transport hazard class(es):		8
Packing group IATA (Cargo):	Packing instruction (cargo aircraft):	855
	Packing instruction (LQ):	Y840
	Packing group:	II
	Labels:	Corrosive
Packing group IATA (Passenger):	Packing instruction (passenger aircraft):	851
	Packing instruction (LQ):	Y840
	Packing group:	II
	Labels:	Corrosive

Lainco SA

UN number: 3267

Proper shipping name:	
ADR/RID:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-sodium in mixture)
IMDG:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-sodium in mixture)
IATA:	CORROSIVE LIQUID, BASIC, ORGANIC, N.O.S. (Metam-sodium in mixture)
Transport hazard class(es):	8 – Corrosive Substances
Packing group:	III
Environmental Hazards: ADR/RID:	Environmentally hazardous substance
IMDG:	Marine Pollutant
ADR/RID	
Hazard identification No.:	80
Classification code:	C7
Tunnel Code:	3 (E)
IMGD	
EmS No.:	F-A, S-B

Precautions concerning fire:

Taminco BV
Extinguishing media

Suitable extinguishing media:
Carbon dioxide (CO_2), dry chemical and water spray

Unsuitable extinguishing media:
Do not use a solid water stream as it may scatter and spread fire. Do NOT use water jet.

Special hazards arising from the substance or mixture:

Specific hazards during fire-fighting:
Thermal decomposition can lead to release of irritating gases and vapours.

Hazardous combustion products:
Nitrogen oxides (NOx)
Carbon oxides
Carbon disulphide
Sulphur oxides

Advice for fire-fighters

Special protective equipment for firefighters:
Wear an approved positive pressure self-contained breathing apparatus in addition to standard firefighting gear.

Further information:
Do not allow run-off from firefighting to enter drains or water courses.

Lainco SA

- In case of fire use the appropriated extinguishing media, chemical powder, carbon dioxide (CO2), foam and sand. Simultaneous use of foam and water on the same surface is to be avoided as water destroys the foam.
- Unsuitable extinguishing media are water pressure jet or water spray. dilution with water may generate toxic fumes. Water pressure jet or water spray. Dilution with water may generate toxic fumes.
- Advice for fire-fighters: Cool the drums/containers by water spraying and in case there is an explosion keep a security distance. Maintain the zone free of people, keeping them at a minimum distance of security (100 meters). Avoid using great volumes of water, in order to minimize the extension of the product. Work always in favour of the wind or in right angle respect to it. Take precautions in case explosions due to the gas production of the product take place. Dilution with water may generate toxic fumes. When exposed to high temperatures and in contact with soil, water and oxygen may give off toxic and flammable fumes. Special protective equipment for fire-fighters: basic protective equipment for fire extinction. Suitable breathing device and protective clothing (suit, gloves of PVC and rubber boots).
- Further information: Do not allow run-off from firefighting to enter drains or water courses. Remains of fire as well as contaminated extinguishing water must be disposed of according to current regulations.

2.4.2 Summary of procedures for destruction or decontamination

Taminco BV
Incineration under controlled conditions in accordance with the criteria laid down in Directive 94/67/EC is the preferred and the most environmental way to safely dispose of the active substance as well as plant protection products and its packaging. The active substance, contaminated materials, or contaminated packaging shall be disposed of through controlled incineration in a licensed incinerator.

The molecular formula of metam-sodium is $C_2H_5NNaS_2$ and of the other variant metam-potassium $C_2H_5NKS_2$.

As both metam variants do not contain more than 1% of halogens, incineration under the following controlled conditions is recommended (refer to Directive 94/67/EC):

- Temperature at least 850°C

Exhaust gases should not exceed:

- 10 mg/m³ total dust as an average on 24 hours
- 10 mg/m³ gaseous and vaporous organic substances, expressed as total organic carbon as an average on 24 hours
- 50 mg/m³ sulphur dioxide (SO_2) as an average on 24 hours

Lainco SA Product:

- Waste identification (Code EWC): 02 01 08* Agrochemical waste containing dangerous substances.
- Prevent the production of waste and analyse possible methods for revaluation or recycling.
- Do not pour under any circumstances down drains nor to the environment.

Contaminated packaging:

- Waste identification (Code EWC): 15 01 10* Packaging containing residues of or contaminated by dangerous substances.
- No residues will remain due to the use of the product if the empty packaging is washed 3 times with water, adding this water to the solution. The package, washed as above mentioned, may be disposed according to the local legislation, in a no contaminant place.
- Do not manipulate the containers nor expose them to heat, sparks or other ignition sources: They may explode.
- Do not remove labels from containers until they have been properly cleaned.

2.4.3 Summary of emergency measures in case of an accident

Taminco BV
General advice:
Show safety data sheet to the doctor in attendance. Call a physician immediately.

Inhalation: Move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Treat symptomatically. If symptoms persist, call a physician.

Skin contact: Wash off with soap and plenty of water. Wash off immediately with plenty of water for at least 15 minutes. Wash contaminated clothing before re-use. In the case of skin irritation or allergic reactions see a physician.

Eye Contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. If easy to do, remove contact lens, if worn. Call a physician or poison control center immediately.

Ingestion: Seek medical advice. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person.

Most important symptoms and effects, both acute and delayed: Health injuries may be delayed. Liver disorders, kidney disorders, rash, redness. Use of alcoholic beverages may enhance toxic effects.

Treatment: General advice for dithiocarbamates. Biomonitoring possible at chronical exposure: determination of TTCA in the urine at the end of the workday/week. Blood testing for delayed effects: liver tests, kidney function, thyroid function.

Accidental release measures

Personal precautions, protective equipment and emergency procedures: Wear appropriate personal protective equipment. Local authorities should be advised if significant spillages cannot be contained.

Environmental precautions: Avoid release to the environment

Methods and materials for containment and cleaning up: Sweep up or vacuum up spillage and collect in suitable container for disposal. Contain spillage, soak up with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and transfer to a container for disposal according to local / national regulations.

Lainco SA
General Advice:
It is recommended to the person that provides first aid measures a previous self-protection.

Inhalation:
Remove the person from the contaminated zone. Put him in rest position, nearly straight, with untied clothing. If necessary, apply artificial respiration.

Skin:
Remove clothing contaminated with the product immediately. Wash it before using again. Wash the affected body zones with abundant water, avoiding rubbing these zones.

Eyes:
Wash the eyes with abundant water at least during 15 minutes. In order to be sure that the washing is complete, the eyelids must remain separated from the eyeball. Do not forget to retire the contact lenses in case the victim had them.

Ingestion:
Do not provoke vomit. Dilute the ingested product by administrating copious amounts of water. If the person is unconscious, lay him side down with the head lower than the rest of the body and the knees bended. Administer activated carbon and a saline type laxative (sodium, magnesium or similar sulphate) with special care in children and persons with hepatic alterations. Keep the victim in rest position. Seek medical assistance in order to perform a gastric lavage. Control of ANTABUSE effect.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1. Methods for the determination of the active substance in the technical material and formulation

Taminco BV:
The metam sodium or metam potassium content in technical concentrate (TK) is determined by CIPAC method method (20/13/M1.3 and 20/SL/M3) (CS_2 evolution method). Since the final formulation is the same as the technical concentrate (TK), the analytical method to determine the metam sodium (or metam potassium) in the TK is also applicable to the representative formulations.

An HPLC-UV method is also available for determination of metam sodium and metam potassium (Taminco BV).

A validated HPLC-UV method is available for the determination of the relevant impurities DMTU and MITC in metam sodium or metam potassium technical concentrate. The same methods are applied in the respective representative formulations.

Lainco SA:
The metam sodium or metam potassium content in technical concentrate (TK) is determined by CIPAC method method 20/SL/M3 (CS_2 evolution method). The same method is used to determine metam sodium in the representative formulation.

A validated method to determine the counter-ion (sodium or potassium) in the technical concentrate is available for Lainco SA. The same method is used in the representative formulation.

A validated HPLC-UV method is available for the determination of the relevant impurity DMTU in metam sodium or metam potassium technical concentrate. HPLC-UV is used to determine DMTU in the representative formulation based on metam sodium.

An HPLC-UV method is available for the determination of the relevant impurity MITC in metam sodium or metam potassium technical concentrate. HPLC-UV is used to determine DMTU in the respective The same method is proposed for the determination of MITC in the representative formulation based on metam sodium.

Data gap (Lainco SA): the method to determine MITC in the TK and PPP should be further validated at a lower level appropriate to batches and specification.

2.5.1.2. Analytical methods for the determination of residues in the different matrices

Methods of analysis which have been submitted to support new studies in relation to the risk assessment have been assessed in accordance with the guidance document SANCO/3029/99 rev.4.

Several methods have been available and therefore submitted as methods for the generation of pre-approval data required for the risk assessment.

Methods that were considered relevant by both applicants for the assessment as well as their validation according to SANCO/3029/99 rev. 4 are reported and discussed in Volume 3 CA-B.5 (B.5.1.2). The acceptability of the overall study is discussed in the volume of the respective expertise area. A summary table of analytical methods used in each risk assessment study is also included in the Vol. 3-CA B5 under the section B.5.1.2.for both applicants.

Adequate methods are usually available and considered “fit for purpose” for the different matrices relevant to the different expertise areas (see Taminco-Table 4.1.2-1 and Lainco-Table 4.1.2-1 under Vol.3 CA - B.5.1.2), with however some few exceptions where the available data do not allow confirming the method was fit for purpose with certainty. The lack of reporting noted in some study reports compared to current study reports and requirements for analytical methods is considered to be usually rather due to the report requirement at the time the old studies were carried out. Some precisions/confirmative information (i.e. chromatograms, etc.) are expected from notifiers for some studies (please refer to Vol. 3 CA-B5).

- For study entitled "*Methyl Isothiocyanate (MITC): An Acute Vapor Exposure Toxicity Study with the Honey Bee*" (2016, report 703H-101), the notifier (Taminco BV) is requested to provide further clarifications on the high variability observed in the analysed exposure concentrations of MITC.

- Lainco SA is requested to provide validation Renolab study 12054-07 R on carrot.

2.5.1.3. Analytical methods for the determination of residues in the different matrices

Methods of analysis have been submitted to support new studies in relation to the risk assessment and have been assessed in accordance with the guidance document SANCO/3029/99 rev.4.

The validation of the method according to SANCO/3029/99 rev. 4 is mainly discussed in Volume 3 B5-CA. The acceptability of the overall study is discussed in the volume of the respective expertise area.
A summary table of analytical methods used in each risk assessment study (both applicants) is also included in the Vol. 3-CA B5 under the section B.5.1.2.

2.5.2 Methods for post control and monitoring purposes

For the assessment of the different analytical methods proposed for monitoring purposes, the current criteria of SANCO/825/00 rev.8.1 were used.

2.5.2.1. Analytical methods for the determination of residues in plant

For the residue definition for monitoring in plant matrices, please refer to Vol. 1 – level 2, B.2.7 and Vol. 1 – level 2, B.2.14. Based on that residue definition, residue analytical methods are required for MITC in food/feed of plant origin.

Matrix	Method	analyte	Confirmatory method	ILV	LOQ	Reference
Food/feed of plant origin (high water, acidic)	Based on BASF 234/2 (GC-MS, 3 monitored ions: m/z 73, 72 and 70)	MITC	Confirmation simultaneous to the primary method (GC-MS: monitoring of 3 m/z ions, no quantitative validation data).	Yes (in high water, acidic matrices)	0.01 mg/kg	[redacted] 2003 – Vol. 3 CA-B.5.2.1 - KCA 4.2/03-Taminco [redacted] 2005 – Vol. 3 CA-B.5.2.1 - KCA 4.2.1/04-Taminco (ILV)
Food/feed of plant origin (high water, acidic)	Based on BASF 234/2 (GC-MS, 3 monitored ions: m/z 73, 72 and 70) (MITC) LC-MS/MS (2 mass transitions) (DMTU)	MITC and DMTU	Confirmation simultaneous to the primary method (GC-MS: monitoring of 3 m/z ions, no quantitative validation data, LC-MS/MS: monitoring of two mass transitions, no quantitative validation data).	Yes (in high water, acidic matrices)	0.01 mg/kg	[redacted] 2012 – Vol. 3 CA-B.5.1.2.5 – KCA 4.1.2/11-Lainco and KCA 4.2/4b-Lainco [redacted] 2015 – Vol. 3 CA-B.5.2.1 – KCA 4.2/4-Lainco (ILV)
Food/feed of plant origin (oily, dry [high starch])	GC-MS/MS (monitoring of 2 mass transitions : 72→ 45 and 72→ 44)	MITC	Confirmation simultaneous to the primary method (GC-MS/MS: monitoring of 2 mass transitions with full validation data).	Yes (in high water, acidic matrices)	0.01 mg/kg	[redacted] 2014* Vol. 3 CA-B.5.2.1 - KCA 4.2/01-Taminco and KCA 4.2/1b-Lainco [redacted] 2014* - Vol. 3 CA-B.5.2.1 - KCA 4.2/02-Taminco and KCA 4.2/1-Lainco (ILV)

* Studies are owned by Taminco and co-owned by Lainco (a letter of co-ownership dated 28 October 2019 has been provided).

<u>High water and acidic matrices:</u>

Taminco BV:

Data gap:
SANCO/825/00 rev.8.1 requires that validation data (specificity, linearity, accuracy and repeatability) are generated for the confirmative ions for GC-MS. However, these data are not available from study 58308 by [redacted] (KCA 4.2/03-Taminco). Only qualitative confirmation (comparison of the ion ratios) data have been reported in the ILV and other residue studies.

Consequently, the method cannot be considered as fully validated in regards to SANCO/825/00 rev. 8.1 due to the absence of sufficient quantitative validation and acceptable validation data for confirmative ions.
Since the method is intended for monitoring, a fully validated method according to SANCO/825/00 rev. 8.1 (including quantitative validation data on confirmative ions of MITC) should be available and should be submitted by Taminco BV.

It could be questioned if the GC-MS/MS method developed for dry and oily matrices couldn't be applied to high water and high acidic matrices in order to try to improve the current recoveries and solve the problem encountered for some crops regarding the confirmation, and generate the required validation data package for confirmation.

Extraction efficiency
No extraction efficiency is required according to SANTE 2017/10632 rev. 3 since no residues above the LOQ were found in the representative crops, lettuce and baby leaf, and no MRL >LOQ is proposed.

Lainco SA:
SANCO/825/00 rev.8.1 requires that validation data (specificity, linearity, accuracy and repeatability) are generated for the confirmative ions for GC-MS and an additional mass transition for LC-MS/MS. However, these data are not available from study [redacted] (KCA 4.2/4b-Lainco). Only qualitative confirmation (comparison of the ion ratios) data have been reported. The applicant mentioned in the course of the assessment that quantitative results are available in the raw data of the study and that the confirmatory data, which was not included in the final report, can be provided upon request.

Data gap: Lainco SA is requested to submit an amendment of study report 1114-01R including the quantitative validation data (specificity/interferences, linearity, accuracy and repeatability) on the confirmative ions for MITC. DMTU is currently not part of the residue definition for monitoring in plants.

Extraction efficiency
N**o statement about extraction efficiency was provided by the applicant, the need to demonstrate or not the extraction efficiency will be pending on the expert's consultation attempted in the residue section.**

2.5.2.2. Analytical methods for the determination of residues in products of animal origin

For the residue definition for monitoring in animal matrices, please refer to Vol. 1 – level 2- B.2.7 and Vol. 1 – level 2 – B.2.14.

Analytical methods are not required as no MRL are proposed.

2.5.2.3. Analytical methods for the determination of residues in soil

For residue definition for monitoring in soil (MITC), please refer to Vol. 1 B.2.14.

For the purpose of renewal, a new monitoring method with a lower LOQ (0.01 mg/kg instead of 0.02 mg/kg) is proposed by Taminco and fully validated.
Lainco proposed its own method.

Matrix	Method	analytes	Confirmatory method	ILV	LOQ	Reference
Soil	GC-MS (three mass fragments)	MITC	Confirmation simultaneous to the primary method (monitoring of three mass fragments)	Not required	0.02 mg/kg	Vol.3 CA B.5.2.3: KCA 4.2/05-Taminco – [redacted] (2002) – V4211
Soil	GC-MS (three mass fragments)	MITC	Confirmation simultaneous to the primary method (monitoring of three mass fragments)	Not required	0.01 mg/kg	Vol.3 CA B.5.2.3: KCA 4.2/06-Taminco – [redacted] (2013) – Study CEMS-6105
Soil	GC-MS (three mass fragments)	MITC	Confirmation simultaneous to the primary method (monitoring of three mass fragments but no validation data generated on the confirmative ions)	Not required	0.01 mg/kg	Vol.3 CA B.5.2.3: KCA 4.2/6-Lainco – [redacted](2013) – Study 12044-01R
Soil	LC-MS/MS (two mass transitions)	DMTU*	Confirmation simultaneous to the primary method (monitoring of two mass transitions but no validation data generated on the confirmative transition)	Not required	0.01 mg/kg	Vol.3 CA B.5.2.3: KCA 4.2/6 (Lainco) – [redacted](2013) – Study 12044-01R

Full risk assessment based on field studies and therefore no Tier I laboratory studies available to derive the lowest endpoint for soil.
(*note: DMTU is however not proposed to be included in the residue definition for monitoring purposes)

Lainco SA:
SANCO/825/00 rev.8.1 requires that validation data (specificity, linearity, accuracy and repeatability) are generated for the confirmative ions for GC-MS and an additional mass transition for LC-MS/MS However, these data are not presented in study by [redacted] (KCA 4.2/6-Lainco) though available from the raw data (please refer to Vol. 3 CA-B.5 – KCA 4.2/06-Lainco). The applicant mentioned in the course of the assessment that these data can be provided upon request.

Lainco SA is requested to submit an amendment of study report 12044-01R including the quantitative validation data (specificity/interferences, linearity, accuracy and repeatability) on the confirmative ions for MITC. DMTU is currently not part of the residue definition for monitoring in soil and further data are deemed to be not necessary.

2.5.2.4. Analytical methods for the determination of residues in water

For residue definition for monitoring in surface and drinking water, please refer to Vol. 1 B.2.14.

For the purpose of renewal, new method is available for the determination of MITC in surface and drinking water, and supersedes the previous one submitted in the frame of the first Annex I inclusion. The method has been fully validated according to SANCO/825/00 rev.8.1 and successfully independently validated and can therefore be recommended for enforcement/monitoring purposes with a validated LOQ of 0.1 µg/L.

Matrix	Method	analyte	Confirmatory method	ILV	LOQ	Reference
Drinking and surface water	Headspace GC-MS (primary method – column DBX-624; m/z 73)	MITC	Independent analytical method – Headspace GC-MS (confirmatory method – column RTX-35 Amine, m/z 73)	Yes	0.1 µg/L*	Vol.3 CA B.5.2.4: KCA 4.2/08-Taminco – [redacted] (2013) – Study CEMS-5667 KCA 4.2/09-Taminco – [redacted] (2014) – Study CEMS-6314 KCA 4.2/10-Taminco – [redacted] (2014) – Study P 3115 G (ILV) Lainco proposed the same monitoring methods as Taminco (KCA 4.2/9-Lainco, KCA 4.2/10-Lainco and KCA 4.2/11-Lainco)

***** The LOQ complies with the trigger limit of 0.1 µg/L set in SANCO/825/00 rev. 8.1 for the drinking and surface water and that LOQ for surface water is < 0.531 µg MITC/L (based on fish study – acute toxicity test with *Oncoryhnchus mykiss*).

2.5.2.5. Analytical methods for the determination of residues in air

For residue definition for monitoring in air (MITC), please refer to Vol. 1 B.2.14.

For the purpose of renewal, no new monitoring methods to determine MITC in air are proposed by Taminco BV. Reference is made to the original monitoring method presented in the initial monograph for Taminco BV and to the same method for Lainco SA, however with both columns inverted for quantitation and confirmation purposes.

The method is considered validated according to SANCO/825/00 rev. 8.1 at ambient conditions and can therefore be recommended for enforcement/monitoring purposes. The validated LOQ of the method (0.25 µg/m^3 or 0.5 µg/m^3) complies with the concentration C (1.2 µg/m^3) calculated based on the current AOEL value of 0.004 mg/kg body weight/day according to SANCO/825/00 rev. 8.1. The AOEL value proposed for renewal is proposed to be kept as initially for the first approval of the active substance.
Method is not validated at 80% relative humidity and 35°C (recoveries < 70%).

Matrix	Method	analyte	Confirmatory method	ILV	LOQ	Reference
Air	GC-NPD	MITC	GC-NPD using another column of different polarity	Not required	0.25 µg/m^3	Vol.3 CA B.5.2.5 KCA 4.2/11-Taminco – [redacted] (2007) – Study V7111
Air	GC-NPD	MITC	GC-NPD using another column of different polarity	Not required	0.5 µg/m^3	Vol.3 CA B.5.2.5 KCA 4.2/13-Lainco – [redacted] (2008) – Study V8209

2.5.2.6. Analytical methods for the determination of residues in body fluids and tissues

For the residue definition in body fluids and tissues monitoring, please refer to Vol. 1 B.2.14.

The following methods are suitable methods for monitoring purposes in body fluids and tissues.

Matrix	Method	analytes	Confirmatory method	ILV	LOQ	Reference
Body fluids (urine and blood plasma)	LC-MS (SIM – m/z 235)*	N-acetyl-S-[(methylamino) carbothioyl] cysteine	Confirmation by LC-MS/MS*	Not required	0.05 mg/L	Vol.3 CA B.5.2.6 KCA 4.2/13 - Taminco – [redacted] (2004) – Study 180682
Body fluids (human urine and blood plasma rat)	LC-MS/MS (3 mass transitions)	N-acetyl-S-[(methylamino) carbothioyl] cysteine	LC-MS/MS: Self-confirmation by monitoring of at least 2 mass transitions	Not required	0.05 mg/L	Vol.3 CA B.5.2.6 KCA 4.2/15 and /16-Lainco – [redacted] (2008) – Studies V8207, V8276
Tissues (liver)	GC-NPD	MITC	Confirmation by GC-NPD with a column of different polarity	Not required	0.1 mg/kg	Vol.3 CA B.5.2.6 KCA 4.2/14-Taminco – [redacted] (2006) – Study A68872

Lainco SA:
Lainco SA initially tried to develop a GC-NPD method to determine MITC in liver but without success (recoveries < 20% - see Vol.3 CA B.5.2.6). As a consequence, the applicant developed a method to determine the acetyl cysteine conjugate of MITC (AMCC) in liver instead, using HPLC-MS/MS. Acceptable recoveries could not be reached with the LC-MS/MS method despite various conditions (see Vol. 3 CA B.5.2.6) and the method should not be recommended for monitoring purposes.
Data gap: New attempts to validate method for determination of MITC in liver should be conducted to accept the method as a monitoring method or access to the study of Taminco BV should be requested.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

More detailed results of the studies are presented in Volume 3, section B.6 (part 1, 2 and 3)

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 17: Summary of ADME

Oral absorption of **metam** and its major metabolite **methylisothiocyanate** (**MITC**) is rapid and important reaching a mean value of 85% (based on excretion in urine - 50% - and expired air - 35% -) for metam and 90-95% for MITC (based on excretion in urine -80-85%- and expired air -10-15% -). Comparing the results from the high and low dose levels, it is clear that metabolism of metam sodium may be saturated at high dose levels resulting in a reduced breakdown of MITC, which is then excreted unchanged via exhaled air. Please refer to Vol.3 B.6 table 6.1.1/01-3: Excretion balance and recovery of radioactive residues after treatment of rat with metam and MITC (Hawkins, 1987).

Tissue distribution is mainly uniform with a slight accumulation in thyroid. Approximately 2.3% of the dose was still detected at 168 h in tissues. Half-life of elimination from plasma ranged from 60.8 to 74.1 hours.

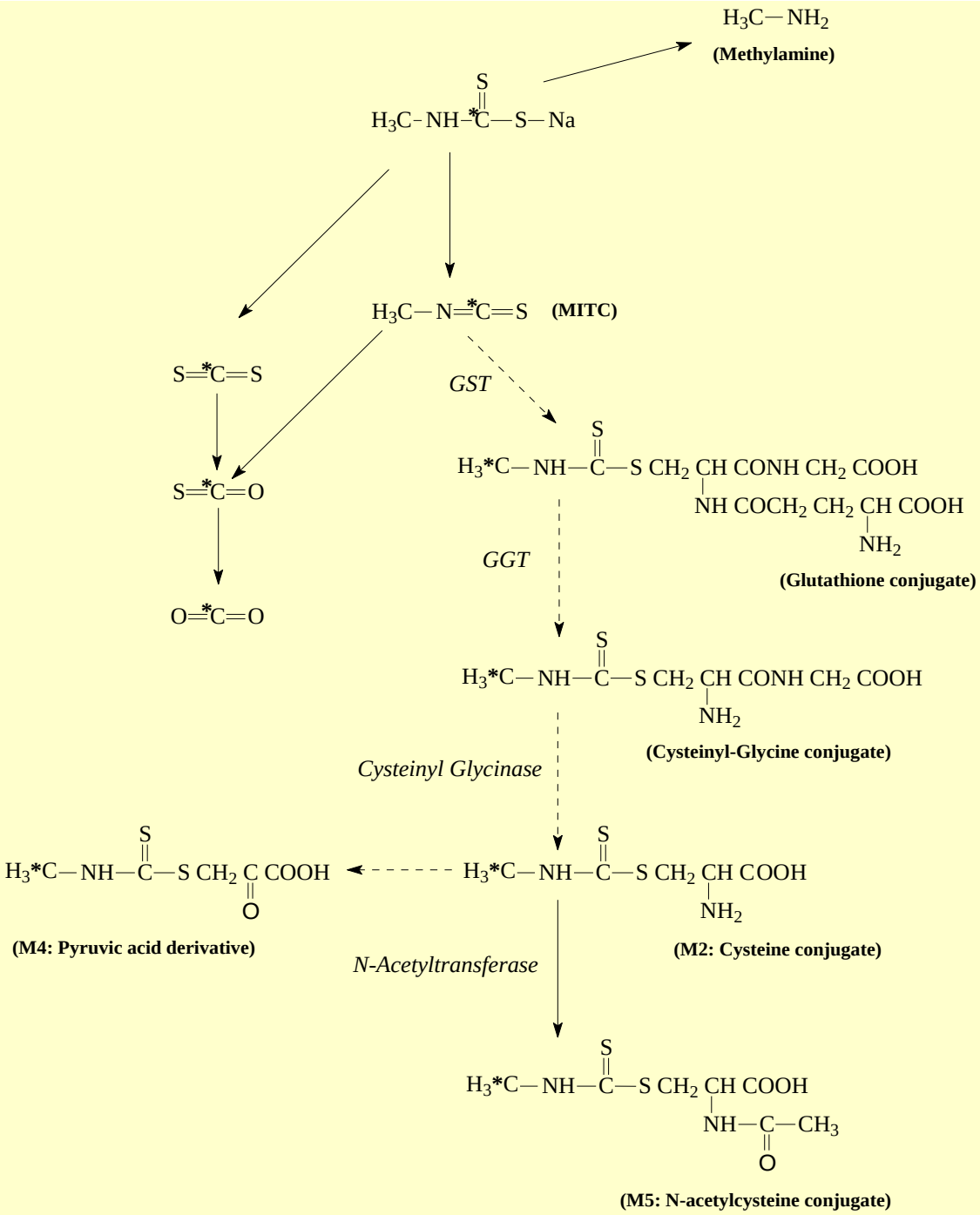
Metabolism is extensive and rapid, suggesting a decomposition of metam into MITC, **carbon dioxide** (**CO_2**), and **carbonyl sulfide** (**COS**). MITC is further conjugated to glutathione (GSH) and excreted in urine while CO_2 and COS are excreted via expired air. By photolysis, MITC may also give rise to **methylisocyanate (MIC)**. The other significant pathway for metam is the release of **carbon disulfide (CS_2),** which could be related to the acidic conditions existent in the stomach of the rat (pH=3.8-5.0) following oral ingestion. From published studies in the open literature, it appears that, although similar metabolites were identified in rats and mice, non-identified part of polar metabolites are more important in mice than in rats suggesting that metabolism of metam and MITC after intraperitoneal administration, is quantitatively and qualitatively different.
Due to chemical instability of [^{14}C]-Metam in buffers/media in which liver S9-fractions, liver microsomal fractions and hepatocyte incubations are incubated, it was decided to perform a comparative *in vitro* metabolism study with MITC. In this [^{14}C]-MITC study using hepatic microsomes from CD-1 mouse, Sprague Dawley rat, New Zealand White rabbit, Beagle dog and human, no human-specific metabolite was identified. The major components detected in this species, M1, M2 and M3, were also detected at comparable or even higher levels in all the other species tested. It is noted that further investigations are currently ongoing (**RMS-BE**: expected 2022), notably to attempt to elucidate the chemical structures of M1, M2 and M3, the major metabolites seen in human microsomes.

Excretion
The majority of the material given was excreted within 24-48 h after administration with minor portions excreted up to 168 h after dosing.

The postulated metabolic pathway for ^{14}C-metam sodium and methyl isothiocyanate in the rat is as follows:
In short, exhaled metabolites were MITC, CS_2, COS and CO_2, while mercapturate, glutathion, cysteine, and acetylcysteine conjugates of MITC were recovered in the urine.

Fig (1): (below) **Postulated metabolic pathway for ^{14}C-metam sodium and methyl isothiocyanate in the rat.**

M2: S-(N-methylthiocarbamoyl)-L-cysteine
M4: S-(N-methylthiocarbamoyl)-pyruvic acid
M5: N-acetyl-S-(N-methylthiocarbamoyl)-L-cysteine



Captions:
GST: Glutathione S-Transferase;
GGT: gamma-Glutamyltranspeptidase;
dashed arrows: precedes probable intermediate; CS2, COS and CO2 recovered in the expired air.

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Oral absorption of **metam** and its major metabolite **methylisothiocyanate** (**MITC**) is rapid and important reaching a mean value of 85% (based on excretion in urine - 50% - and expired air - 35% -) for metam and 90-95% for MITC (based on excretion in urine -80-85%- and expired air -10-15% -). Comparing the results from the high and low dose levels, it is clear that metabolism of metam sodium may be saturated at high dose levels resulting in a reduced breakdown of MITC, which is then excreted unchanged via exhaled air.

Tissue distribution is mainly uniform with a slight accumulation in thyroid. Approximately 2.3% of the dose was still detected at 168 h in tissues. Half-life of elimination from plasma ranged from 60.8 to 74.1 hours.

Metabolism is extensive and rapid, suggesting a decomposition of metam into MITC, **carbon dioxide** (**CO_2**), and **carbonyl sulfide** (**COS**). MITC is further conjugated to GSH and excreted in urine while CO_2 and COS are excreted via expired air. By photolysis, MITC may also give rise to **methylisothiocyanate (MIC)**. The other significant pathway for metam is the release of **carbon disulfide (CS_2),** which could be related to the acidic conditions existent in the stomach of the rat (pH3.8-5) following oral ingestion. From published studies in the open literature, it appears that, although similar metabolites were identified in rats and mice, non-identified part of polar metabolites are more important in mice than in rats suggesting that metabolism of metam and MITC after intraperitoneal administration, is quantitatively and qualitatively different.
Due to chemical instability of [^{14}C]-Metam in buffers/media in which liver S9-fractions, liver microsomal fractions and hepatocyte incubations are incubated, it was decided to perform a comparative in vitro metabolism study with MITC. In this [^{14}C]-MITC study using hepatic microsomes from CD-1 mouse, Sprague Dawley rat, New Zealand White rabbit, Beagle dog and human, no human-specific metabolite was identified. The major components detected in this species, M1, M2 and M3, were also detected at comparable or even higher levels in all the other species tested. It is noted that further investigations are currently ongoing (**RMS-BE**: 2022), notably to attempt to elucidate the chemical structures of M1, M2 and M3, the major metabolites seen in human microsomes.

Excretion
The majority of the material given was excreted within 24-48 h after administration with minor portions excreted up to 168 h after dosing.

The postulated metabolic pathway for ^{14}C-metam sodium and methyl isothiocyanate in the rat is as follows: exhaled metabolites were MITC, CS_2, COS and CO_2, while mercapturate, glutathion, cysteine, and acetylcysteine conjugates of MITC were recovered in the urine.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Metam					
Acute oral toxicity EPA FIFRA 81-1/ equivalent to OECD TG 401 (1987) GLP compliant No batch number or expiry date of the test substance	Rat, Sprague-Dawley m/f 5/ group/ sex	**Metam-sodium** Batch n°: not specified Purity: 533 g/L	640 mg/kg bw (m/f) 800 mg/kg bw (m/f) 1000 mg/kg bw (m/f) 1600 mg/kg bw (m/f) Single application (gavage)	LD_{50} = 896 mg/kg bw (m/f) 870 mg/kg bw (m) 924 mg/kg bw (f) ***Acute Oral Tox. 4 (H302: Harmful if swallowed)***	[redacted]; 1991 DRAR : **B.6.2.1/01**
Acute oral toxicity OECD TG 423 (2002) GLP compliant No deviations	Rat, Wistar m/f 3/ group/ sex	**Metam-potassium** Batch n°: V814/2207KF Purity: 54.7 %, aqueous solution	<u>Step 1</u> 2000 mg/kg bw (3f) <u>Step 2</u> 200 mg/kg bw (m/f) Single application (gavage)	LD_{50} = 1000 mg/kg bw ***Acute Oral Tox. 4 (H302: harmful if swallowed)***.	[redacted], 2002 DRAR : **B.6.2.1/02**
MITC					
Acute oral toxicity OECD TG 401 (1981) GLP compliant stability of the dose preparations not given. No age of the animals given at the day of administration. Number of air changes during housing of the animals lacking.	Rat, Wistar m/f 5/ group/ sex	**MITC** Batch n° 6205MK Purity 98% Vehicle: olive oil	68.1 mg/kg bw (m/f) 100 mg/kg bw (m/f) 147 mg/kg bw (m/f) 215 mg/kg bw (m/f) Single application (gavage)	LD_{50} = approx.. 147 mg/kg bw (combined) ***Acute Oral Tox. 3 (H301 : toxic if swallowed)***	[redacted], 1986 DRAR: **B.6.8.1.1/01**

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
No data available				
MITC				
Case report	MITC	Acute MITC poisoning caused by drinking 50 g of diluted MITC.	Retrosternal burning, epigastric pain and vomiting were the first signs of intoxication followed by deep coma with pulse 98/min and blood pressure 90/60 mm Hg. Further, a complete loss of all reflex and motor activity. Eight hours after the intoxication with MITC the patient died and necrotic mucosa was observed in the oesophagus, stomach, and proximal part of the duodenum.	[redacted] (1981) DRAR : **B.6.9.3/03**

Table 20: Summary table of other studies relevant for acute oral toxicity with metam

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute oral toxicity Not guideline compliant Not GLP compliant	Metam sodium, Vehicle PBS	SD Rats were dosed *in vivo* with metam sodium (0 or 2 mmol/kg bw, 3♂ rats/group, corresponding to 0 or 260 mg/kg bw. blood was drawn via the tail vein from each rat 24h before dosing and was used to obtain baseline ALT and AST levels. Rats were fasted 18h before and 5h after dosing. After 24 h, another 1 mL blood was taken from each rat for determination of ALT and AST.	↑ALT and AST in treated animals relative to controls, but no increased levels of γGT or bilirubin, indicators of bile duct injury. Histopathology: centrilobular infiltration of mononuclear inflammatory cells in all 3 animals treated with metam sodium. The inflammation was uniform in all lobules and hepatocytes also exhibited signs of cell injury and death. Conclusion: severe hepatotoxic effect of metam after a single oral administration at 265 mg/kg bw/d, → STOT-SE1, (effects oral rat at the guidance value of ≤300 mg/kg b.w.), H370 «Causes damage to the liver via ingestion».	Thompson (2002) – published paper (Toxicol. Sci. 70 269-280) DRAR: **B.6.2.1/03**
Acute neurotoxicity study Equivalent to OECD TG 424 (1997) Doses too high. Muscle histological section not performed. Sections including hippocampus and retina not included. GLP compliant	Metam Sodium Batch n° not reported Purity 43.15% Vehicle: deionised water	Single gavage application to groups of Crl:CD® BR rats at doses of 50, 750 and 1500 mg/kg bw. 12 animals/sex/group were used for the control, 50 and 750 mg/kg bw groups. 16 animals/sex/ group were used for the 1500 mg/kg bw group.	Mortality: 0 mg/kg bw: none 50 mg/kg bw: none 750 mg/kg bw: none 1500 mg/kg bw: 5/16 males; 3/16 females Significant bw decrease/loss at 750 and 1500 mg/kg bw (day 0-7) Total motor and ambulatory activity were decreased from the lowest dose onwards.	[redacted] (1993) [redacted] DRAR: **B.6.7.1/01**

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Metam (incl. -sodium and -potassium):

[redacted] (1991), [redacted]

The acute oral toxicity of metam sodium was investigated in Sprague-Dawley rats. Test substance, suspended in water was orally administered by gavage at a dosage of 640, 800, 1000 or 1600 mg/kg bw

There were deaths following a single oral dose of Metam Sodium among male rats dosed at 800 mg/kg bw and above and among female rats dosed at 1000 mg/kg bw and above. Deaths occurred within 4 hours of dosing until day 2. No abnormalities, which related to administration of the test substance, were observed at the terminal autopsy.

Under the conditions of this study the acute LD50 value of Metam Sodium were estimated to be 870 mg/kg bw for males and 924 mg/kg bw for females (896 mg/kg bw combined).

[redacted] (1993), [redacted]

In the study from Lamb (1993) the objective was to perform an overall neurotoxicological evaluation of the Crl:CD® BR rats after single dose of metam sodium at dose levels of 50, 750 and 1500 mg/kg bw, including Functional Observational Battery (FOB) and motor activity assessments, at the time-of-peak effect (approximately 45 minutes post dosing) and on days 7 and 14. A neuropathological examination centred on the central and peripheral nervous system was performed in the control and 1500 mg/kg bw groups.

The following parameters and end points were evaluated in this study: viability, clinical signs, body weights, functional observational battery (FOB), motor activity evaluations, organ weights, gross necropsy observations, and neurohistopathological evaluations.

Five males and three females died following dose administration with 1500 mg/kg bw metam sodium. All other male and female rats survived until scheduled euthanasia. Several clinical signs and severe effects on body weight were observed in the 750 and 1500 mg/kg bw dose groups.

Total motor and ambulatory activity were decreased from the lowest dose onwards, suggesting a specific effect of metam sodium on the nervous system.

[redacted] (2002), [redacted]

Acute oral toxicity of metam potassium (54.7% aqueous solution) was tested in female and male Wistar rats. The test item was suspended in de-ionised water and administered as a single oral dose to overnight fasted (approximately 17 hours) Wistar rats at the doses of 200 (G1 - females and males) and 2000 (G2 females) mg/kg bw and the rats were observed for 15 days post treatment.

The treatment was initiated with female rats at the dose of 200 mg/kg bw. Lethargy was observed on day 1 of test item administration in female rats. There were no pre -terminal deaths and no gross necropsy changes observed. As per Annex 1 b of the guideline (OECD TG 423), the dose of 200 mg/kg bw was administered to male rats. As in the females, the males presented lethargy on day 1 of test and lethargy persisted in one male rat on day 2. No pre-terminal deaths and no gross necropsy changes were observed.

In consequence, the dose of 2000 mg/kg bw was considered and administered to female rats.

In G2 group, lethargy was observed in one rat, lethargy and convulsions in other rat and another rat was lethargic and recumbent. All the 3 female rats died. The acute oral LD_{50} of Metam Potassium aqueous solution in Wistar rats is 1000 mg/kg bw as per the Interpretation of results based on Annex 3b of the OECD TG 423.

Thompson (2002) – published paper (Toxicol. Sci. 70 (2002) 269-280)

SD Rats were dosed *in vivo* with metam sodium (0 or 2 mmol/kg bw, 3♂ rats/group, dissolved in PBS, corresponding to 0 or **260 mg/kg bw** blood was drawn via the tail vein from each rat 24 h before dosing and was used to obtain baseline ALT and AST levels. Rats were fasted 18 h before and 5 h after dosing. After 24 h, another 1 mL blood was taken from each rat for determination of ALT and AST. Analysis of the serum for markers of liver injury indicated significant elevations for both ALT and AST in treated animals relative to controls, but no increased levels of γGT or bilirubin, indicators of bile duct injury were seen. Upon examining the liver sections using light microscopy, infiltration of mononuclear inflammatory cells into the region surrounding the central hepatic vein occurred in all 3 of the animals treated with metam sodium. The inflammation observed was uniform in all lobules examined and hepatocytes in the centrilobular region also exhibited signs of cell injury and death. Thus, oral administration of a single dose of metam sodium produced centrilobular hepatocyte necrosis accompanied by inflammation and leakage of AST and ALT. Due to the severe hepatotoxic effect of metam after a single oral administration at 260 mg/kg bw the substance qualifies for a classification according to the CLP: STOT-SE1, (effects oral rat at the guidance value of ≤300 mg/kg bw), with H-statement H370 «Causes damage to the liver via ingestion»).

The histopathological adverse finding was supported by a significant 1.6×-increase of ALT compared to the controls.

The study from [redacted] on acute oral toxicity (77/062, DRAR **B.6.2.1/04**) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness this study is nevertheless summarised. The results of this study were in line with the results of the studies relied upon. Therefore, they were not considered for the hazard and risk assessment and are not further used in the CLH dossier for classification purposes.

MITC:

[redacted] (1986), [redacted]

The acute oral toxicity of MITC was investigated in male and female rats (5 animals/sex/group) of the Wistar strain according to the acute toxic class method. Test substance, suspended in olive oil, was orally administered by gavage to each animal at a dosage of 68.1, 100, 147 or 163 mg/kg bw. Mortality and clinical signs were recorded during the subsequent 14 days.
Mortality occurred in the dosages of 147 and 215 mg/kg bw.
In the dose groups 147 mg/kg and 215 mg/kg unspecific signs of poisoning occurred, like dyspnoea, apathy, staggering, paresis, twitching, piloerection, exsiccosis, salivation and poor general state.
Necropsy findings in animals that died were: general congestive hyperaemia, some animals were cadaverous; diagnosis was not possible.
Necropsy findings in animals that were sacrificed were: slight intra-abdominal adhesions and thickening of the wall with white-yellow coat in the forestomach.
No abnormalities or mortality was observed in the 68.1 and 100 mg/kg bw groups.
Thus, the LD50 is 163 mg/kg and 147 mg/kg bw for males and females, respectively (155 mg/kg bw combined).

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

Metam (incl. -sodium and -potassium):

The guidance on the application of the CLP criteria (Regulation (EC) 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for the classification of acute toxicity *via* the oral route. Under the conditions of this study, The most sensitive LD_{50} value of metam sodium for oral toxicity was found to be 870 mg/kg bw for males ([redacted] 1991), which is below the trigger value of 2000 mg/kg bw. For metam potassium a LD_{50} of 1000 mg/kg bw was observed, which is below the trigger value of 2000 mg/kg bw.

According to the guidance of the application of the CLP criteria, the LD_{50} values for both salt forms are, determined within the present study, is in the concentration range of 300 and 2000 mg/kg bw triggering classification of metam sodium for acute oral toxicity, hazard category 4. Thus, classification of metam sodium and metam potassium for acute oral toxicity category 4 is proposed.

MITC:

The guidance on the application of the CLP criteria (Regulation (EC) 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for the classification of acute toxicity via the oral route. Under the conditions of this study, the lowest LD_{50} value of MITC for oral toxicity was found to be 147 mg/kg bw for females, which is below the trigger value of 2000 mg/kg bw. According to the guidance of the application of the CLP criteria, the LD_{50} value, determined within the present study, is in the concentration range of 50 and 300 mg/kg bw triggering classification of MITC for acute oral toxicity, hazard category 3. Thus, classification of MITC for acute oral toxicity, category 3 is proposed.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Metam (incl. -sodium and -potassium):

Classified – Acute oral toxicity, Category 4 (H302: harmful if swallowed)
The LD_{50} value is between 300-2000 mg/kg bw, therefore the acute oral toxicity point estimate (ATE) is 500 mg/kg bw.
MITC:

Classified – Acute oral toxicity, Category 3 (H301: toxic if swallowed)
The LD_{50} value is between 50-300 mg/kg bw, therefore the acute oral toxicity point estimate (ATE) is 100 mg/kg bw.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Metam					
Acute dermal toxicity EPA FIFRA 81-2/ comparable to OECD TG 402 (1981) GLP Batch number and expiry date of the test substance were not provided. A limit test with a dose of 2 g/kg bw was tested. As mortality was observed a limit test is not indicated when strictly following the guidance. Nevertheless, taking into account the effects on the remaining animals (no major clinical signs, no mortality) the results from the limit test are considered acceptable for risk assessment and classification purposes.	Rat, Sprague-Dawley m/f 5/ group/ sex	**Metam-sodium** Batch n° not specified Purity: 533 g/L Vehicle: water	Single application 2000 mg/kg bw (m/f) 24 hours, occlusive	> 2000 mg/kg bw ***No classification***	[redacted]; 1991 [redacted] DRAR : **B.6.2.2/01**
Acute dermal toxicity OECD TG 402 (1981), GLP, no deviations	Rat, Wistar, m/f, 5/ group/ sex	**Metam potassium** Batch n°:: V814/2207KF Purity: 54.7% Vehicle: water	Single application 1.57 mL/kg bw (2000 mg/kg bw)	> 2000 mg/kg bw ***No classification***	[redacted], 2002 [redacted] DRAR : **B.6.2.2/02**
MITC					
Acute dermal toxicity OECD TG 402 (1981), GLP, animal age upon administration, number of air changes during housing and in-life dates were not provided. Homogeneity and stability of the dose preparations was not reported.	Rat, Wistar m/f 5/ group/ sex	**MITC** Batch n° 6205MK Purity 98% Vehicle: olive oil	Single application 215 mg/kg bw (m/f) 1000 mg/kg bw (m/f) 1470 mg/kg bw (m/f) 2150 mg/kg bw (m/f) 24 hours semi-occlusive	LD_{50} = 1290 mg/kg bw (combined) ***Acute Dermal Tox. 4 (H312: harmful in contact with skin)***	[redacted], 1987 [redacted] DRAR: **B.6.8.1.1/02**

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: No data are available				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: No data are available				

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Metam (incl. -sodium and -potassium):

[redacted] (1991), [redacted]

The acute dermal toxicity of metam sodium was investigated in Sprague-Dawley rats. The test substance was dermally applied to each animal at a dose of 2000 mg/kg bw for 24 hours.

A dose of 2000 mg/kg bw of Metam Sodium under 24-hour occlusive conditions caused moderate erythema and oedema in some rats of each sex. Staining of the dose site and/or hard white areas of skin as well as scab formation occurred in three of ten individuals.

The acute dermal LD_{50} value of Metam Sodium was greater than 2000 mg/kg bw for both male and female rats.

[redacted] (2002), [redacted]

The acute dermal toxicity of Metam Potassium (54.7% aqueous solution) was investigated in five male and five female Sprague-Dawley rats. The undiluted test item at the dose of 2000 mg (1.57 mL)/kg bw was applied evenly on the prepared area of skin and covered with a cotton gauze and secured with an adhesive tape wrapped around torso. After a 24 hour contact period with the skin, the unabsorbed test item was removed by washing and the rats were observed for 15 days.

Erythema was observed in one male rat and in all female rats on day 3 and 4, post application. Erythema persisted along with scale formation in all the female rats on day 5 and continued in two female rats till day 6 followed by peeling/desquamation of skin was observed on day 7. Peeling/desquamation of skin was also observed in three female rats on day 6. The skin was normal from day 8 onwards.

There were no toxic signs, pre-terminal deaths and gross necropsy changes. The acute dermal minimum lethal dose of Metam Potassium aqueous solution in Wistar rats is greater than 2000 mg/kg bw.

The study from [redacted]on acute dermal toxicity ([redacted], DRAR **B.6.2.2/03**) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness this study is nevertheless summarised. The results of this study were in line with the results of the studies relied upon. Therefore, they were not considered for the hazard and risk assessment and are not further used in the CLH dossier for classification purposes.

MITC:

[redacted] (1987), [redacted]

MITC was tested for its potential acute hazard after 24-hour percutaneous exposure. Five ♂ and 5 ♀ Wistar rats each were administered 215, 1000, 1470 and 2150 mg/kg bw by application of the test substance emulsion (diluted in olive oil) to dorsal/dorsolateral parts of the trunk, covering the application site with a semi-occlusive dressing for 24 hours. After removal of the dressing the site was rinsed with warm water. Mortality and clinical signs were recorded during a subsequent 14-day observation period.

Toxic signs were dyspnoea, apathy, staggering, tremors and poor general state. As local findings erythema, oedema and scaling were observed.

Mortality occurred in the dose groups 1000, 1470 and 2150 mg/kg bw. Necropsy findings in animals that died were: general congestion, bloody ulcerations in the glandular stomach. In sacrificed animals no abnormalities were detected.

Thus, the LD_{50} in rats is 1290 mg/kg bw.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Metam (incl. -sodium and -potassium):

The guidance on the application of the CLP criteria (Regulation (EC) 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for acute dermal toxicity classification. Under the conditions of these studies the LD_{50} value of Metam Sodium and Metam Potassium for dermal toxicity was found to be > 2000 mg/kg bw. Therefore, no classification for acute dermal toxicity is proposed.

MITC:

The guidance on the application of the CLP criteria (Regulation (EC) 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for acute dermal toxicity classification. Under the conditions of this study, the LD_{50} value of MITC for dermal toxicity was found to be 1290 mg/kg bw for both genders, which is below the trigger value of 2000 mg/kg bw. According to the guidance of the application of the CLP criteria, the LD_{50} value, determined within the present study, is in the concentration range of 1000 and 2000 mg/kg bw triggering classification of MITC for acute dermal toxicity, hazard category 4. Thus, classification of MITC for acute dermal toxicity, category 4 is proposed.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Metam (incl. -sodium and -potassium):

Not classified – Conclusive but not sufficient for classification.

MITC:

Classified – Acute dermal toxicity, Category 4 (H312: harmful in contact with skin)

The LD_{50} value is between 1000-2000 mg/kg bw, therefore the acute dermal toxicity point estimate (ATE) is 1100 mg/kg bw.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations[1] if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**
Metam					
Acute inhalation toxicity EPA FIFRA 81-3, equivalent to OECD TG 403 (1981), No batch number, the vehicle of the test substance, air changes per hour and the photoperiod specified. No raw data for clinical signs. GLP compliant	Rat, Sprague-Dawley m/f 5/group/sex	**Metam sodium** Batch n° not specified, Purity: 533 g/L (aerosol, MMAD 3.1 ± 2.10 µm/3.0 ± 2.04 µm/3.2 ± 2.05 µm/3.2 ± 2.27 µm) Vehicle: not reported	1.23 mg/L 2.46 mg/L 3.03 mg/L 3.15 mg/L 4 h inhalation (whole body)	LC_{50} = 2.54 mg/L (m/f) 2.20 mg/L (m) 2.95 mg/L (f) ***Inhalation Acute Tox 4 (H332: harmful if inhaled***	[redacted]; 1992 DRAR: **B.6.2.3/01**
Acute inhalation toxicity OECD TG 403 (1981), GLP, no deviations. GLP compliant	Rat, Wistar m/f 10/group/sex	**Metam sodium** Batch n° ZH 130 585 Purity: 42.2%; (aerosol, MMAD 2.6 ± 3.0 µm)	6.8 mg/L 4 h inhalation (nose only)	LC_{50} > 6.8 mg/L ***No classification***	[redacted].; 1986 DRAR: **B.6.2.3/02**
Acute inhalation toxicity OECD TG 403 (1981), GLP, no deviations. GLP compliant	Rat, Wistar m/f 5/group	**Metam potassium** Batch: V814/2207KF Purity: 54.4 %	2.98, 3.21 and 3.90 mg/L 4 h inhalation (aerosol)	LC_{50} = 3.11 mg/L (m) 3.03 mg/L (f) 3.04 mg/L (m/f) ***Inhalation Acute Tox 4 (H332: harmful if inhaled***	[redacted] 2002 DRAR: **B.6.2.3/03**
MITC					

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity OECD TG 403 (1981). Three days instead of at least 5 days acclimation period of the animals, number of air changes, duration of light and dark cycle not given, the in-life dates were not reported and the stability of the dose preparations were not analysed, whole body inhalation chamber instead of the preferred nose-only exposure, no determination of the particle-size distribution, exposure temperature higher than 22±3°C (highest temperature 26°C). GLP compliant	Rat, Sprague-Dawley m/f 5/group/sex	**MITC** Batch n° 051897 Purity 98%	0.282 mg/L (m/f) 0.496 mg/L (m/f) 0.570 mg/L (m/f) 0.628 mg/L (m/f) 0.786 mg/L (m/f) 1.64 mg/L (m/f) 4 h inhalation (whole-body).	LC_{50} = 0.54 mg/mL ***Acute Inhalation Tox. 2 (H330: toxic if inhaled)*** ***Specific Target Organ Toxicity - Single Exposure 3, H335: may cause respiratory irritation***	[redacted] 1981 [redacted] DRAR: **B.6.8.1.1/03**

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
Acute Metam Sodium Poisoning Caused by Occupational Exposure at a Flower Farm	Metam Sodium	In October 2016, an outbreak of vomiting, fainting, and diarrhea occurred among employees of a flower farm in central Uganda. Among the farm's 562 employees, 110 cases were identified while 27 employees were hospitalised.	No deaths were reported. Vomiting, fainting, and diarrhea occurred	Nakubulwa, S. (2018) DRAR: **B.6.9.3/01**
MITC				
No data available				

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam: no data available				
MITC				
Acute neurotoxicity study OECD TG 424 (1997) GLP compliant	**MITC** Batch n° 51198PJV, Purity 99.7%	Rat, Crl:CD(SD) Males and females 12/group/sex (main group) 6/group/sex (satellite group) Vehicle: none, vapour exposure 60, 120, 240 mg/m^3 6 hours, whole body (Preliminary test): 1/10 ♀ found dead at 300 mg/m^3)	Main test: NOAEL (systemic/ neurotoxicity): < 60 mg/m^3 NOAEL (local effects in nasal cavity/lung) <60 mg/m^3 No mortality occurred	[redacted] (2011) [redacted] DRAR: **B.6.8.1.6.1**

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Metam (incl. -sodium and -potassium):

Two studies were carried out to investigate the potential of metam sodium and one study was carried out to investigate the potential of metam potassium for acute inhalation toxicity.

[redacted] (1986), [redacted]
Wistar rats were exposed by the inhalation route to **metam sodium** in air for 4 hours to nose only at an actual concentration of 6.8 mg/L.
There was no mortality during the 14 day post administration observation period. Body weight reductions were observed after one week for males, but all animals had recovered by the end of the 14 day observation period. The bodyweight of females were slightly reduced over the whole observation period. No abnormalities were observed at the necropsy.
Under the conditions of this study the acute LC_{50} value of metam sodium was determined to be greater than 6.8 mg/L (purity 42.2%) in male and female rats.

[redacted] (1992), [redacted]
Sprague-Dawley rats were exposed by the inhalation route to **metam** sodium in air (whole body) for 4 hours with target concentrations between 1.23 and 3.15 mg/L.
Mortality was noted in the 3 highest dosing groups, which occurred within the first 3 days of the observation period. Clinical signs detected were partial closing of the eyes, wetness around the eyes, wet snout, reddening of the ears, feet and tail, exaggerated respiratory movements and the adoption of a hunched or prone posture during the exposure and exaggerated and noisy respiration, wet and stained body fur, peripheral vasodilation and lethargy during the observation period. Food and water consumption was decreased after exposure for 3 or 9 days respectively.
Lung weights to bodyweight ratios were higher than the control values in all decedent rats.
Rats treated with 3.15, 3.03 and 2.43 mg/L metam sodium showed histopathological lung changes progressing to bronchiolar epithelial necrosis with inflammatory exudate. Some rats treated with the highest doses, and all rats treated with 1.23 mg/L metam sodium survived to termination and showed an increased incidence of prominent alveolar macrophages over the control group.
Under the conditions of this study the acute LC_{50} value of Metam Sodium 510 G/L was determined to be 2.20 mg/L in ♂ and 2.95 mg/L in ♀ rats (2.54 mg/L for combined sexes).

[redacted] (2002), [redacted]
The acute inhalation toxicity of **metam potassium** (54.7% aqueous solution) was determined in Wistar rats by exposure to an aerosol of the undiluted test item generated by stainless steel atomisers at 1.2 kg/cm² pressure and injection rates of 2.0, 3.0 and 4.0 mL/min. for G1, G2 and G3 groups respectively. An analytically determined average concentration of 2.98, 3.21 and 3.90 mg a.s./L of chamber air was achieved for G1, G2 and G3 groups respectively. The rats housed in special rat restrainers, were continuously exposed to the aerosol by head and nose exposure for four hours in a 0.5 m³ stainless steel whole body exposure dynamic state inhalation chamber. Toxic signs observed were lethargy, tremors, slight salivation and dyspnoea in all the groups and ataxia was observed in one male rat additionally in G2 group. These toxic signs were seen predominantly on day 1 soon after exposure and on days 2 to 5. The pre-terminal deaths were 40, 70 and 90% in G1, G2 and G3 groups respectively and all the deaths occurred either during exposure period or the next day (day 2).
The calculated acute inhalation (4 hours) LC_{50} value of metam potassium aqueous solution in ♂ Wistar rats is 3.11 mg a.s./L of chamber air. The calculated acute inhalation (4 hours) LC_{50} value of metam potassium aqueous solution in ♀ Wistar rats is 3.03 mg a.s./L of chamber air. The calculated acute inhalation (4 hours) LC_{50} value of metam potassium aqueous solution in Wistar rats (combined sex) is 3.04 mg a.s./L of chamber air with 95 confidence limits of 2.73 and 3.38.

The study from [redacted] on acute inhalation toxicity ([redacted], DRAR **B.6.2.3/04**) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness this study is nevertheless summarised in the MCA 5 dossier (KCA 5.2.3/04). The results of this study were in line with the results of the studies relied upon. Therefore, they were not considered for the hazard and risk assessment and are not further used in the CLH dossier for classification purposes.

MITC:
[redacted] 1981 [redacted]
In an acute inhalation toxicity study, groups of young adult Sprague-Dawley rats (5/group/sex), were exposed by the inhalation route to MITC (98% purity) in air for 4 hours to whole body at a target concentrations between 0.282 and 1.64 of g/m³. Mortality and clinical signs were recorded during the subsequent 14 days.
With one exception, all rats that died or would have died and were killed to prevent further suffering, died within 2 days of exposure. The one exception died on the third day following exposure. All animals died in the three highest dose groups.
Clinical signs observed were marked irritation and included closure of the eyes, lacrimation and peripheral vasodilation in all exposed rats and excessive salivation and the adoption of a hunched posture by a majority of rats. During the observation period peripheral vasodilation and wet jaws due to the excessive salivation persisted for some hours following exposure. All surviving rats had swollen abdomens on the first day of observation. Rates were heard in all rats at this time

and in the majority of surviving rats during days 3-4 of observation. Opacity of the eyes with observed in all surviving rats in groups 2 and 6 and in 4 male rats in group 5 on day one of observation. Other occasional signs included poor grooming, staining around head or snout and lethargy.
Both sexes lost weight between the day of exposure and the first day of the observation period. A marked increase in the lung weight to bodyweight ratio was observed in all rats that died. In surviving rats, the ratio was minimally elevated (~29%) in male rats.
The stomach and intestines were distended with gas, and the lungs were congested. In some cases, red hepatisation was evident. Microscopic findings were congestion, oedema, bronchiolitis, interstitial pneumonitis, intra-alveolar haemorrhage, disorganisation and mitotic activity in the bronchiolar epithelium and dedifferentiation of the bronchiolar epithelium (lung) and focal necrosis (liver).
Thus, the acute LC_{50} value of MITC administered in the form of vapour was determined to be 0.54 g/m³ (± 0.015 g/m³; corresponding to 0.54 mg/L or 540 mg/m³) in ♂ and ♀ rats.

[redacted] (2011), [redacted]
In the acute neurotoxicity study rats were treated by inhalation with up to 240 mg/m³ for 6 h. No mortality occurred. In the preliminary study 1/10 females died at a concentration of 300 mg/m³. These results support the effects observed in the acute inhalation study.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

Metam (incl. -sodium and -potassium):
The guidance of the application of the CLP criteria (Regulation (EC) No 1272/2008) gives a LC_{50} cut-off value of 5 mg/L for dust / mist to trigger classification for acute inhalation toxicity. Under the conditions of the study from [redacted] (1986), metam sodium had an LC_{50} value of >6.8 mg/L and as hence, no classification for acute inhalation toxicity is proposed. On the other hand, after whole body exposure metam sodium in the study from [redacted] (1992) an LC_{50} value of 2.54 mg/L was observed for both genders combined, which is below the trigger value of 5 mg/L. For metam potassium a combined LC_{50} of 3.04 mg/L was observed after head/nose exposure for 4 h ([redacted], 2002). According to the guidance of the application of the CLP criteria, the LC_{50} value, determined within the present study, is in the concentration range of 1.0 and 5.0 mg/L triggering classification of metam sodium and metam potassium for acute inhalation toxicity, hazard category 4.
Thus, classification of metam-sodium and -potassium for acute inhalation toxicity category 4 is proposed.

MITC:
The guidance of the application of the CLP criteria (Regulation (EC) 1272/2008) gives a LC_{50} cut-off value of 20 mg/L for vapours to trigger classification for acute inhalation toxicity. LC_{50} values between 0.5 and 2 mg/L for testing of vapours require a classification in **Category 2 (H330: Fatal if inhaled),** which is indicated for MITC with a LC_{50} of 0.54 mg/L (vapour). Based on the known corrosive properties and the observation of corrosive effects in the lung/respiratory tract, additional labelling with **"EUH071: Corrosive to the respiratory tract"** is indicated.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Metam (incl. -sodium and -potassium):
Classified – Acute inhalation toxicity, Category 4 (H332: Harmful if inhaled)
The LC_{50} value is between 1-5 mg/L mist, therefore the acute inhalation toxicity point estimate (ATE) is 1.5 mgL.
It is also observed that the gaseous decomposition of metam sodium into MITC under acidic conditions* justifies the need for the additional mention: **EUH031** 'Contact with acids liberates toxic gas'
It is noted that the substance already has this phrase in the existing Annex VI entry of metam-sodium.

*See Vol.3 B.6, B.6.9.4/05 (Bretaudeau-Deguigne, 2011, Metam sodium intoxication: the specific role of degradation products – methyl isothiocyanate and carbon disulphide – as a function of exposure).
The release of CS2 as a minor metabolite was also demonstrated in the TK studies, and also in field studies (see [redacted], 2006 (reference to product DAR).

MITC:
Classified – Acute inhalation toxicity, Category 2 (H330: Fatal if inhaled)
The LC_{50} value is between 0.5-2 mg/L vapour, therefore the acute inhalation toxicity point estimate (ATE) is 0.5 mg/Lbw.
Additional labelling suggestion: **EUH071**: Corrosive to the respiratory tract

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
Metam					
Acute skin irritation study OECD 404 (2015), GLP, no concentration of the treatment dose, batch number and stability of the test substance, acclimation period, CAS number	Rabbit, New Zeeland White m/f 3/group/sex	**Metam sodium** Batch n° not reported Purity: 533 g/L	24 h dermal application 0.5 mL ≡ 266.5 mg	**Corrosive** - necrotic lesions accompanied by very slight to slight oedema (day 2) - necrosis with fissuring and associated haemorrhage (day 8) - well-defined erythema accompanied by slight to moderate oedema (day 1) Mean scores: Erythema: 3.33, 2, 1.33, 3.33, 4,4 not fully reversible after 14 days ***Skin Corrosive 1 (H314: causes severe burns and eye damage)***	[redacted].; 1991 DRAR: **B.6.2.4/02**
Acute skin irritation study OECD 404 (1981), GLP, no concentration of the treatment dose, no batch information	Rabbit, Vienna White m/f 1 m/ 2 f per dose	**Metam Fluid** (**Metam sodium**) Batch n° not reported Purity: 560 g/L	0.5 ml (280 mg) 4 h dermal occlusive treatment Observations at 24h,48h, 74h, and 8d	**Corrosive** - necrotic lesions at day 8 (2/3 animals) - Erythema and oedema not reversible after 8 days Mean scores: Erythema: 3 for animal 1, 2, 3 Oedema: 2, 2.3, 2.3 for animal 1, 2, 3 ***Skin Corrosion 1 (H314: causes severe skin burns and eye damage)***	[redacted], 1982 DRAR: **B.6.2.4/01**
MITC					
Acute skin irritation study OECD 404 (1981), GLP, concentration of the treatment dose, CAS number and stability of the test substance, animal age at dosing, air changes per hour and the in life dates not provided.	Rabbit (White Vienna) Males /Females 3/group	**MITC** Batch n° 6205MK Purity 98%	4 h dermal occlusive treatment 0.5 mL (presumably from a 98%-purity stock substance resulting in 490 mg test substance per application)	**Corrosive** After application, severe signs of irritation were observed which persisted up to 72 hours. At this time superficial necrosis was seen in all animals. As reversibility could not be expected the study was terminated after 72 hours. Mean score (24-72 h): Erythema: 4 (score 4 in 6/6 animals) Oedema: 3.3 (score ≥2.3 in 4/6 animals) ***Skin Corrosion 1 (H314: causes severe skin burns and eye damage)***	[redacted], 1986 DRAR: **B.6.8.1.1/04**

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
Occupational disease observation	Vapam	Incidence of contact dermatitis in 1973/1974	17 patients showed dermatitis following Vapam exposure	Jung H.D. (1973) DRAR: **B.6.9.2/01**
MITC				
Case report	MITC	Observations on nine patients with occupational dermatitis from methylisothiocyanate.	MITC causes primarily a toxic dermatitis, but as a strong sensitiser it induces sensitisation as well. In one patient a MITC acid burn on one foot and leg caused systemic poisoning from absorption with reversible damage to the liver parenchyma.	Richter G. (1980) DRAR: **B.6.9.3/04**
Case report	MITC	Methyl isocyanate and similar compounds were tested for their ability to cause contact dermatitis. Methyl isothiocyanate (MITC), formed by hydrolytic decomposition, was identified to be the main allergen.	In this report allergic reactions were noted for methyl-isothiocyanate. Methyl mustard oil is formed by hydrolytic decomposition and was identified as the main allergen causing strong skin reactions like contact dermatitis.	Schubert H. (1978) DRAR: **B.6.9.3/05**

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
No data available				
MITC				
No data available				

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Metam (incl. -sodium and -potassium):

[redacted]: 1991 [redacted]

Metam Sodium was applied to New Zeeland White rabbits to investigate its dermal irritation potential. 0.5 mL of the test substance was held in contact with the skin under semi-occlusive conditions for 4 hours. After removal of the test substance, the treated skin was examined for signs of irritation on day 1 to 5 and 14.

Persistent well-defined to severe dermal irritation was produced by a 4 hour semi-occlusive application of metam sodium. The mean score over 24, 48, and 72 hours was ≥ 2.3 and ≤ 4.0 for erythema for 4 of 6 animals resulting in a classification for skin irritation. The mean scores for oedema were below 2.3 for all animals. 2/6 animals were sacrificed due to severe skin lesions. Necrosis was observed in 4/6 animals. In 2/6 animals skin reactions were fully reversed within 10 days. In conclusion, metam sodium is considered corrosive to the skin.

[redacted] 1982 [redacted]

In a second study 1 male, 2 females Vienna White rabbits were exposed to 0.5 mL test substance (metam sodium) for 4 h under occlusive conditions ([redacted], 1982). Non-reversible erythema and oedema reactions were observed in any animal. The mean scores over 24, 48, and 72 h were 3, 3, and 3 for erythema and 2, 2.3, 2.3 for oedema, respectively. Skin reactions were not reversible within 8 days (last day of observation). Necrosis was observed (surface and full-thickness) in 2/3 animals. In conclusion, **metam sodium** is considered corrosive to the skin.

MITC:

[redacted], 1986 [redacted]

MITC was applied to 6 White Vienna rabbits (3/sex) to investigate its dermal irritation potential. 0.5 mL of the unchanged and undiluted test substance was held in contact with the skin (exposed area: approximately 2.5 cm × 2.5 cm) under semi-

occlusive conditions for 4 hours. After removal of the test substance, the treated skin was rinsed with lutrol and lutrol/water (1:1) and examined 30 – 60 min (4 h time point), 24 h, 48 h and 72 h after application for clinical signs of irritation. If there were clinical signs of necrosis, these were confirmed by gross-pathological examination after incision of the skin.

MITC showed severe signs of irritation, which persisted up to 72 hours to rabbit skin. Mean score (24-72 h) was 4 (score 4 in 6/6 animals) for erythema and 3.3 (score ≥2.3 in 4/6 animals) for oedema. Superficial necrosis was observed in all animals, which was not reversible and led to termination of the study after 72 hours.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Metam (incl. -sodium and -potassium):

The guidance on the application of the CLP criteria (EC 1272/2008) requires that the mean irritation score is ≥ 2.3-≤4 for erythema/eschar or for oedema in at least 2 of 3 tested animals at 24, 48 and 72 hours after patch removal before classification as a skin irritant is triggered. In addition, a substance is classified as corrosive if destruction of skin tissue, namely, visible necrosis, in at least 1 tested animal after exposure up to 4 hour duration is observed.

Under the conditions of the studies mentioned above, superficial and full-thickness necrosis was observed in several animals, being not reversible. Due to severe skin reactions 2 animals were killed in the study of [redacted] (1991). According to the guidance of the application of the CLP criteria, the obtained results trigger the classification of metam sodium for skin corrosion/irritation in at least hazard category 1C. Nevertheless, as no information is available for exposure periods of 3 min or 1 h a final sub-categorisation is not possible. Thus, classification of metam sodium for skin corrosion/irritation, category 1 is proposed.

The irritating properties are caused by the metam acid and/or the main metabolite MITC, and classification is corrosive cat.1. As there are no toxicological arguments to consider that primary irritating potential of the K-salt would meaningfully be different from that the of the Na-salt, RMS considers that a pragmatic approach could be taken, also avoiding to request new irritation/ corrosivity studies *in-vivo*. In general, during the first EFSA-peer review, it was agreed that from toxicological point of view, no distinction should be made between the two variants, as no meaningful difference would be expected from the counter-ions Na or K after dissociation, also taking into account that MITC as the mean metabolite, may drive the risk assessment.

MITC:

The guidance on the application of the CLP criteria (EC 1272/2008) requires that the mean irritation score is ≥ 2.3-≤4 for erythema/eschar or for oedema in at least 4 of 6 tested animals at 24, 48 and 72 hours after patch removal before classification as a skin irritant is triggered. In addition, a substance is classified as corrosive if destruction of skin tissue, namely, visible full thickness necrosis, in at least 1 tested animal after exposure up to 4 hour duration is observed.

Under the conditions of this study, superficial necrosis was observed in all animals at 72 h, which was not reversible and led to termination of the study after 72 hours. According to the guidance of the application of the CLP criteria, the obtained results trigger the minimum classification of MITC for skin corrosion/irritation, hazard category 2. However, due to the strong effects observed and assuming that the effects are not reversible a worst case approach is applied. It can be assumed that the effects may get worse and the superficial necrosis may develop to full thickness necrosis. Thus, classification of MITC for skin corrosion/irritation, category 1 is proposed.

Classification in Cat 1A can be excluded as no necrosis was observed during an observation period of 1 h. As necrosis was observed at 72 h after a 4 h treatment period and no information was available at 60 min after end of exposure it cannot be excluded that exposure of up to 60 min may also cause necrosis in the animals. Thus, classification in Category 1B is feasible as a worst case approach. Nevertheless, according to the guidance on CLP classification no distinct classification is possible, as no data after a 60 min exposure is available. Therefore, classification as Skin Corrosive Category 1 is considered most appropriate.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Metam (incl. -sodium and -potassium):

Classified – skin Corr. 1, H314: skin corrosion/irritation, Category 1 (H314: causes severe skin burns and eye damage)

MITC:

Classified – skin Corr. 1, H314: skin corrosion/irritation, Category 1 (H314: causes severe skin burns and eye damage)

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
Metam					
Acute eye irritation study Equivalent to OECD 405 (1987), GLP, no batch data	Rabbit, New Zealand White m/f 3/sex and group	**Metam-sodium** Batch: Not reported Purity: 510 g/L	0.1 mL undiluted test substance (510 g/L)	**Not irritating** Cornea: no effects Iris: no effects Conjunctivae, redness: 0.33, 0.66, 0.66, 1, 0.66, 1.66 (fully reversible within 4 days) ***No classification triggered, but logically classified as Skin Corrosion 1 (H314: causes severe skin burns and eye damage)***	[redacted], 1991 [redacted] DRAR: **B.6.2.5/01**
Acute eye irritation study According to OECD 405 (1987), GLP, no deviations	Rabbit, New Zealand White m 3/sex and group	**Metam potassium** Batch n°: V814/2207KF Purity: 54.7 % w/w	0.1 mL undiluted test substance	**Not irritating** Cornea: no effects Iris: no effects Conjunctivae, redness: no effects ***No classification triggered, but logically classified as Skin Corrosion 1 (H314: causes severe skin burns and eye damage)***	[redacted], 2002 [redacted] DRAR: **B.6.2.5/02**
MITC: no specific studies are available					

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam: no data available				
MITC				
Acute Skin Irritation/ Corrosivity study OECD 404 (2002) Concentration of the treatment dose, CAS number and stability of the test substance,	MITC Batch n°: 6205 MK Purity: 98%	Rabbit, White Vienna, Males and Females 3/group 0.5 mL exposure on skin for 4 hours, semi-occlusive	**Corrosive** After application, severe signs of irritation were observed which persisted up to 72 hours. At this time, superficial necrosis was seen in all animals. As reversibility could not be expected the study was terminated after 72 hours. -Mean score: 4 (in 3/3 animals)	[redacted]. 1986 [redacted] DRAR: **B.6.8.1.1/04**

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
animal age at dosing, air changes per hour and the in life dates GLP compliant				
Acute Inhalation Toxicity study OECD 403 (2009) GLP compliant	MITC Batch n°: 051897 Purity: 98%	Rat, Sprague-Dawley Males and Females 5/group/sex Vehicle: none, vapour exposure 282-1640 mg/m³ 4 hours, whole body	LC_{50}: 0.54 g/m³ (± 0.015 g/m³); corresponding to 0.54 mg/L Clinical signs/observations: Closure of the eyes Opacity	[redacted] 1981 [redacted] DRAR: **B.6.8.1.1/03**
Acute neurotoxicity study study OECD 424 (1997) GLP compliant	MITC Batch n°: 56198PJV Purity; 99.7%	Rat, Crl:CD(SD) Males and Females 12/group/sex (main group) 6/group/sex (satellite group) Vehicle: none, vapour exposure 60, 120, 240 mg/m³ 6 hours, whole body	Clinical signs/observations: Closure of the eyes No microscopic findings on the eye	[redacted] [redacted] (2011) [redacted] DRAR: **B.6.8.1.6/01**

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Metam (incl. -sodium and -potassium):

[redacted]., 1991 [redacted]

0.1 mL metam sodium was instilled into the eyes of New Zeeland White rabbits to investigate its eye irritating potential. The eyelids were held gently together for one second before releasing. The contralateral eye remained untreated and served as a control. Examination (cornea opacity, iris lesion, conjugative redness/chemosis and discharge) of the eyes was made after 1 hour and 1, 2, 3, 4 and 7 days after instillation.

Four animals showed conjunctival redness with means of 1, and two animals showed means of 2. A diffuse crimson-red colouration of the conjunctivae was observed in four animals. Chemosis (mean score: 1) was observed in 3/6 animals. No effects on cornea or iris were observed. All reactions had resolved two, three or four days after instillation. In conclusion, slight irritating effects were observed, which were fully reversible and for which no classification is warranted.

[redacted], 2002 [redacted]

Metam potassium was instilled into the eyes of 3 male New Zeeland White rabbits to investigate its eye irritating potential. A volume of 0.1 mL of the undiluted test item was instilled into the conjunctival sac of the left eye.

The right eye served as the reference control. The effects on the conjunctiva, cornea and iris were scored by the evaluation method of Draize at 1, 24, 48 and 72 hours post-instillation.

The results indicate that the test item metam potassium aqueous solution is 'non-irritant' to the eyes of New Zealand White rabbits based on the scores at 24 hours under the testing conditions adopted.

On the basis of this study alone, metam potassium does warrant classification as being an eye irritant.

MITC:

No data are available from specific eye irritation studies. Nevertheless, data from an acute inhalation and neurotoxicity study show at least an eye irritating effect of MITC. A skin irritating study with MITC in rabbits revealed corrosive effects on the skin which is also relevant for classification for the endpoint "eye irritation/serious eye damage".

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Metam (incl. -sodium and -potassium):

The guidance on the application of the CLP criteria (EC 1272/2008) lists the following criteria for classification as an eye irritant (Category 2):

A positive response in at least 2 of 3 tested animals of:

Corneal opacity	≥ 1 and/or
Iritis	≥ 1 and/or
Conjunctival redness	≥ 2 and/or
Conjunctival oedema (chemosis)	≥ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within 21 days.

These criteria were not met for any animal in the study. Therefore, no classification for serious eye damage/eye irritation is triggered by the available eye irritation studies.

MITC:

No data from specific *in vitro* or animal studies on eye irritation are available, which may be indicative of the potential of MITC to cause serious eye damage/eye irritation in humans. Nevertheless, data from an acute inhalation and neurotoxicity study show at least an eye irritating effect of MITC in rats. Furthermore, MITC is classified as "corrosive to the skin, Cat. 1" which intrinsically includes a classification for eye damage (H314)".

No additional classification for eye irritation/damage is necessary.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Metam (incl. -sodium and -potassium):

Due to the classification of metam for skin irritation/corrosion in Category 1 (H314: causes severe skin burns and eye damage), effects on the eyes and respective classification are already covered. When classified as Skin Corr. 1, the risk of severe damage to eyes is considered implicit.

Classified – Irreversible effects on the eye/serious damage to eyes, Category 1, (H318: Causes serious eye damage)

MITC:

Due to the classification of metam for skin irritation/corrosion in Category 1 (H314: causes severe skin burns and eye damage), effects on the eyes and respective classification are already covered. When classified as Skin Corr. 1, the risk of severe damage to eyes is considered implicit.

Classified – Irreversible effects on the eye/serious damage to eyes, Category 1, (H318: Causes serious eye damage)

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Metam					
No data available					
MITC					
No data available					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
No data available				
MITC				
No data available				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
No data available on respiratory sensitisation, but evidence of RADS caused by MITC needs to be considered (see below)				
MITC				
No data available on respiratory sensitisation, but evidence of RADS caused by MITC needs to be considered (see below)				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Metam (incl. -sodium and -potassium): No data are available.

MITC: No data available.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

Metam (incl. -sodium and -potassium):
No data from animal studies are available, which may be indicative of the potential of metam to cause respiratory sensitisation in humans.

MITC:
No data from animal studies are available, which may be indicative of the potential of metam to cause respiratory sensitisation in humans.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

Metam (incl. -sodium and -potassium):
No classification warranted.

MITC:
No classification warranted.

Remark RMS:
There was ample epidemiological evidence of both skin irritation and respiratory tract irritation subsequent to accidental release or agricultural application of metam. In all cases, the major breakdown product MITC was considered the main

cause of the distress in the affected persons, in the view of the observed symptoms. In general, the respiratory tract symptoms emerged quite rapidly (24h) after exposure, although cases of delayed symptoms (<1 week) were also noted. In the spill incident, persistent respiratory findings (up to 15 months post-spill in adults, and >3 months in children) occurred, which were diagnosed as **chemically induced asthma**, and more specifically **RADS** (Reactive Airway Dysfunction Syndrome). Likewise, skin irritation emerged rapidly in persons coming into contact several hours with water potentially contaminated with MITC, while persons who changed clothes immediately after water contact did not complain of skin rashes.
Both airway irritation and dermatoses were not associated with the sensitising potential of MITC. In addition, the involvement of other breakdown products or other environmental contaminants was not ruled out.

More details are reported at length in vol.3 of the DRAR, more specifically in section B.6.9.8 Overview of clinical cases and poisoning incidents and epidemiological data.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: Summary table of animal studies on skin sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Metam					
Skin sensitisation study OECD TG 406 (1981), GPMT data on purity, batch, stability were lacking; challenge was performed with 2 concentrations per animal GLP compliant	Albino guinea pig, Dunkin/Hartley Females Test: 20 Control: 20	**Metam sodium** Batch n°: not reported Purity: 533 g/L Vehicle: water	Induction: Intradermal: 5% Topical: 2.5% Challenge: 0.1 and 1%	Sensitising to skin - Erythema anterior site: 0 (1/20), 1 (6/20) and 2 (13/20) - Erythema posterior site: 0 (4/20), 1 (9/20) and 2 (7/20) - Oedema anterior site: 0 (5/20), 1 (8/20) and 2 (7/20) - Oedema posterior site: 0 (11/20), 1 (7/20) and 2 (2/20) ***Skin sensitiser 1 (M&K) (H317: may cause an allergic skin reaction)***	[redacted]; 1991 [redacted] DRAR: **B.6.2.6/01**
Skin sensitisation study OECD TG 406 (1981), GPMT no deviations GLP compliant	Guinea pig, Test: 20 Control: 10	**Metam potassium** Batch n°: V814/2207 KF Purity: 54.2% Vehicle: water	Induction: Intradermal: 5% Topical: 25% Challenge 1/2: 1% Challenge 3: 0.5%	Based on challenge 3: Test substance: 2/20 positives (Score 1), 9/20 (Score 0.5) Control: 0/10 positives (Score 1), 7/10 (Score 0.5) Not sensitising to skin (≤10% positive response) ***No classification***	[redacted] 2002 [redacted] DRAR: **B.6.2.6/02**
MITC					

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Skin sensitisation study OECD TG 406 (1992) stability (expiry date), CAS number, air changes per hour and animal age at dosing. GLP compliant	Guinea pig (Pirbright White, Dunkin Hartley HOE DHPK [SPF-LAC]) Females Test: 10 Control: 10	**MITC** Batch n° 6205MK Purity 98% Vehicle: olive oil DAB 8	Induction: Intradermal: 2% in olive oil in Freund's adjuvant/water (1:1) Topical: 2% in olive oil 1st challenge: 0.5% in olive oil 2nd challenge : 0.5 in olive oil	**Positive** *1st challenge:* 7/20 slight erythema, 4/20 distincterythema and slight oedema, 1/20 distinct erythema *2nd challenge*: 7/20 slight erythema, 2/20 distinct erythema and slight oedema, 4/20 distinct erythema ***Skin sensitiser 1 (M&K)(H317 may cause an allergic skin reaction)***	[redacted], 1986 DRAR **B.6.8.1.1**

Table 37: Summary table of human data on skin sensitisation

Type of data/ report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam / MITC				
Clinical cases	Vapam (30% Metam-Na)/ MITC	16 persons indicative of a contact dermatitis due to Vapam in agricultural workers handling Vapam-treated potatoes were reported. Seven of them were further investigated using the patch test technique. The concentrations of a Vapam formulation ranged from 1.5-10% as well as airborn aerosols from a 10% preparation	All 7 patients reacted to the applied concentrations and the authors concluded that the reactions seen were not only toxic reactions but also of the allergic type. Even airborne vapours in a room did cause such reactions (airborne contact dermatitis). While under practical field conditions the skin reaction in a ♀ agricultural worker was noted 3 weeks after start of exposure, skin findings as soon as 2d after re-exposure were noted one year later. MITC generation was assumed to play an important role in the development of the dermatitis (toxic and allergic). Presumably MITC was responsible for toxic and allergic contact dermatitis in agricultural workers. Even vapours in a closed room were sufficient to cause skin reactions in sensitised persons	Jung and Wolff, 1970.
	Metam/ MITC	New cases of dermatitis in agricultural workers of areas where Metam–Na was used for potato treatment. The primary cause was probably the formation of MITC when the compound was in contact with sweating wet skin. Patch tests MITC (0.1% aqueous) were tested in patients.	Positive skin reactions in patch tests were reported for MITC, but no detailed information was given. It was only specified that allergic reactions were (i) exposure times (symptoms?) of 1 week of more, (ii) strong focal flare-up reaction in the previous contact areas when patch testing on the back, (iii) itching/burning sensation on the skin when in contact with Metam-Na in the air in the laboratory and (iv) strong incorporation of radionuclides only in Metam-Na eczema patients as a specific reaction in the lymphocyte-transformation test.	Schubert, 1978.
	Metam/ MITC	Nine cases of occupational dermatoses related to MITC. The patients have been handling MITC generating soil disinfectants like dazomet and Metam-Na. They reported several cases of similar skin reaction in colleagues, who had not seeked dermatological advice.	Although the patients were only exposed to MITC and MITC-generating biocides for a short time (few hours to few days), 8/9 showed a strong positive reaction (++ or +++) to Vapam® (Metam-Na) even when the test at a non-irritant concentration in water of 0.05% was repeated 1 year later. According to the author the workers were handling a 10% aqueous Vapam® or Nematin® formulation containing 32.7 or 29.5% MITC, respectively. It was concluded that MITC can cause both strong skin irritation and sensitisation. Based on the exposure information given by the patients, MITC must be regarded as a potent skin sensitiser in human.	Richter, 1980.

Type of data/ report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	Metam/ MITC	Human patch tests. (i) In patients, sensitised to Afungin®, a local human antimycotic drug (3,5-Dibenzylperhydro-1,3,5-thiadiazin-2-thion), closely related to Dazomet, and related compounds releasing either benzylisothiacyanate (BITC) or beta-phenylethyl-isothiacyanate. (ii) In addition, patients sensitised to Nematin® (Na methyl-dithiocarbamate, or Metam-Sodium) were tested to closely related structures including MITC. The patients were tested under occlusive conditions for 24h (Gothatest ®) at specified concentrations who varied from test substance to test substance.	All skin reactions 24, 48 and 72 hours after the test were evaluated as positive. Patients (results were given for the 48h-period) sensitised to Afungin® reacted positively to the benzyl-derivates but not phenylethyl-derivates, suggesting that the side chain might influence the result of the test. Patients sensitised to Metam-Na (0.01% aqueous preparation) reacted positively (4/4 patients) and also 1 patient tested for MITC (0.01% in petrolatum) was positive. Dazomet was also tested (1% in petrolatum) and 1/4 patients sensitised to Metam-Na reacted positive. The methyl moiety of MITC would be the relevant allergenic structure, while the isothiocyanate group would be responsible for the haptene conjugation due to its high affinity to proteins. No further explanation was provided to substantiate the latter hypothesis.	Würbach, 1983.
MITC				
Clinical cases	See above			

Conclusion: sensitising potential of Metam/ MITC in humans:

Several clinical cases indicate that MITC and metam cause severe dermal irritation (occasionaly evolving to bullous lesions with scaling or exfoliation, or in association with oedema). There was also ample evidence of contact sensitisation, occasionally even after inhalation of MITC vapours. Metam, which is known to hydrolyse to MITC in mildly acidic wet conditions, had similar proprieties, and the cases were no sensitising effect was observed on patch testing were restricted to conditions were the compound was suspended in organic/oily vehicles, were the substance could not dissociate to MITC, probably explaining the improved tolerance.
As MITC is a strong skin sensitiser, and may readily be formed after hydrolysis of metam, it is evident to classify metam itself for its potential sensitising properties under conditions of normal handling and use, but also in animal tests metam has been proven a skin sensitiser, increasing the WoE of involvement of MITC-generating substances in skin sensitisation.

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
N.a.				
MITC				
N.a.				

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Metam (incl. -sodium and -potassium):
[redacted] (1991), [redacted]
Skin sensitisation potential of metam sodium was assessed in a GPMT. Female guinea-pigs were assigned into two experimental groups of twenty animals each. The testing concentration of metam sodium was selected based on toxicity and irritancy observed in a preliminary study. The main study consisted of an induction phase with intradermal injections (5% v/v in water; 5% with Freund's complete adjuvant in water, 50:50) and topical application and a challenge phase with topical application (0.5% and 1% v/v in distilled water) . Dermal reactions in the test animals elicited by the challenge application were compared with the findings simultaneously obtained in the control animals and scored for erythema and oedema.
Based on the results of this study, metam sodium has shown a skin sensitisation potential.

[redacted] (2002), [redacted]
Skin sensitisation potential of metam potassium was assessed in the Magnusson-Kligman sensitisation assay using guinea-pigs. Preliminary irritation testing was performed on 12 animals to determine appropriate concentrations of the test substance that could be used for both intradermal and topical induction as well as topical challenge.

The first induction phase involved six intradermal injections into the suprascapular area of each of 20 guinea pigs. These doses were comprised of pairs of injections of the test substance, the test substance combined with Complete Freund's Adjuvant and Adjuvant alone. A sham control group (ten animals) was maintained under the same conditions and received injections like the test group with distilled water instead of the test substance.
Approximately one week later, the second phase of induction was conducted. The test substance or distilled water was applied topically for a period of 48 hours to the area encompassing the intradermal injection sites. Approximately one hour after the topical induction patches were removed, all animals were scored for erythema.
Approximately two weeks later, a challenge dose of the test substance mixed with distilled water was applied for 24 hours to a naive site on each test and sham control animal. Approximately 24 and 48 h after challenge patch removal the animals were scored for a sensitisation response.
The results of challenge were inconclusive and a re-challenge was performed. A new set of naive control animals was placed on test. Approximately one week after the primary challenge, a re-challenge dose was applied for 24 hours to a naive site on each test and naive animal. Approximately 24 and 48 hours after re-challenge patch removal the animals were scored for a sensitisation response.
The results of the first re-challenge were also inconclusive and a second re-challenge was necessary. A new set of naive control animals was placed on test. Approximately one week after the first re-challenge, a 2nd re-challenge dose of the test substance (at a lower concentration) was applied for 24 h to a naive site on each test and naive animals. Approximately 24 and 48 hours after the 2nd re-challenge patch removal the animals were scored for a sensitisation response. Two out of 20 animals of the test group showed positive reactions (score 1) whereas no positive reactions were observed in the control animals (0/10).
Based on the results of this study, metam potassium is not considered to be a contact skin sensitiser as only 10% positive reactions were observed, which is not sufficient for classification purposes.
The positive response observed in the historical positive control validation study with α-hexylcinnamaldehyde, technical grade, 85%, validated the test system used in this study.

The study from [redacted] on skin sensitisation properties of metam ([redacted], DRAR **B.6.2.6/03**) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness this study is nevertheless summarised. The results of this study were in line with the results of the studies relied upon. Therefore, they were not considered for the hazard and risk assessment and are not further used in the CLH dossier for classification purposes.

MITC:
[redacted] (1986), [redacted]
The substance MITC was tested for its sensitising effect on the skin of the guinea-pig in the maximisation test based on Magnusson and Kligman. After intradermal induction (2%) distinct erythema and oedema were observed at the injection sites of the control animals and the test animals, which were applied with Freund's adjuvant/water (1:1). The control animals, which were applied with olive oil DAB 8, exhibited distinct erythema.
After the percutaneous induction, necrotic skin changes and distinct oedema (caused by the intradermal induction) were observed in the test group. Olive oil DAB 8, which was applied to the control animals, caused incrustation (partially open) in addition to distinct erythema and oedema.
After the first challenge 7/20 test animals exhibited slight erythema, 4/20 test animals showed distinct erythema in addition to slight oedema and in 1/20 test animals distinct erythema was observed. Control group 1 did not show any skin changes. After the second challenge 7/20 test animals exhibited slight erythema, 2/20 test animals showed distinct erythema in addition to slight oedema and in 4/20 test animals distinct erythema was observed. Control groups one and two did not show any skin changes.
Olive oil OAB 8 which was applied as a vehicle did not cause any skin reactions after the first and the second challenge either in the control animals or in the test group.
Under these test conditions and following the results described above MITC has a sensitising effect on the skin of the guinea pig.

As MITC is a strong skin sensitiser, and may readily be formed after hydrolysis of metam, it is evident to classify metam itself for its potential sensitising properties under conditions of normal handling and use. Human case studies further support the causal association between exposure to Metam/MITC and skin sensitisation.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

Metam (incl. -sodium and -potassium):
According to the guidance on the application of the CLP criteria (EC 1272/2008), a sensitising potential of a substance is identified, if response of at least 30% of the animals is considered as positive in a Guinea pig maximisation test. In the current study with metam sodium, the 19/20 positive reactions were noted. Hence, positive reactions were observed for >30% of treated animals, which is above the trigger value of 30% for the classification for skin sensitisation, hazard category 1. Thus, classification of metam sodium for skin sensitisation, category 1 is proposed.
The overall response in the [redacted], GPMT assay (1991) is 19/20 (95%) for the anterior flank (1% challenge concentration) and 17/20 (85%) for the posterior flank (0.5% challenge concentration), noted at 72h.

Under CLP, a sub-categorisation as Cat.1A is possible if for the GPMT assay ≥30 % responding at ≤0.1 % intradermal induction concentration or ≥60 % responding at concentrations >0.1 % to ≤1 % intradermal induction concentration (see 3.4.2.2.3.2. Non human data, table 3.4.3).
However, since the intradermal induction concentration was as high as 5% v:v as the solely tested dose, and no lower induction concentrations were tested for positive outcomes after challenge of 85-95%, a further subcategorisation remains impossible. When Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B; therefore, metam-sodium is considered a dermal sensitiser Cat.1.The results from the study with metam potassium do not support the classification as a skin sensitiser, however the reliability of the study performed with metam potassium is questionable with regards to erythema formation in control and treated groups.
Following a weight of evidence metam sodium and metam potassium are considered skin sensitisers cat 1.

MITC:
According to the guidance on the application of the CLP criteria (EC 1272/2008), a sensitising potential of a substance is identified, if response of at least 30% of the animals is considered as positive in a Guinea pig maximisation test. In the current study, the 12/20 (1st challenge) and 13/20 (2nd challenge) positive reactions were noted at 48h. Hence, positive reactions were observed for >30% of treated animals, which is above the trigger value of 30% for the classification for skin sensitisation, hazard category 1.
Under CLP, a sub-categorisation as Cat.1A is possible if for the GPMT assay ≥30 % responding at ≤0.1 % intradermal induction concentration or ≥60 % responding at concentrations >0.1 % to ≤1 % intradermal induction concentration (see 3.4.2.2.3.2. Non human data, table 3.4.3).
However, since the intradermal induction concentration was as high as 2% v:v as the solely tested dose, and no lower induction concentrations were tested for positive outcomes after 2nd challenge of 60%, a further subcategorisation remains impossible. When Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B; therefore, MITC is considered a dermal sensitiser Cat.1.
As the concentration of 2% was the lowest concentration tested for intradermal induction no distinction between sub-categorisation in either 1A or 1B was possible.
Thus, classification of MITC for skin sensitisation, category 1 is proposed.

As MITC is a strong skin sensitiser, and may readily be formed after hydrolysis of metam, it is evident to classify metam itself for its potential sensitising properties under conditions of normal handling and use. Human case studies further support the causal association between exposure to Metam/MITC and skin sensitisation.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Metam (incl. -sodium and -potassium):
Classified – skin sensitisation, Category 1 (H317: May cause an allergic skin reaction)

MITC:
Classified – skin sensitisation, Category 1 (H317: May cause an allergic skin reaction)

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance	Dose levels duration of exposure	Results	Reference
Metam				
No data available. Not considered necessary, since a study on MITC (the main metabolite) was conducted				
MITC				
Neutral Red Uptake Assay OECD TG 432 no deviations GLP compliant	**MITC** Batch n° STBH5869 Purity >99%	1000, 316, 100, 31.6, 10.0, 3.16, 1.00 and 0.316 µg/mL. Incubation: 60 minutes Irradiation: 21 minutes, concentration of 5 J/cm² UV-A Post-incubation for 20-24 h	IC_{50} value without UV-A: 3.36 µg/mL IC_{50} with UV-A: 2.04 µg/mL PIF value: 1.65. **No phototoxicity**	[redacted], 2019 20221896 DRAR: **B.6.8.1.1/06**

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

Metam (incl. -sodium and -potassium):
No data are available from humans. As metam is a solid substance and no liquid hydrocarbon, aspiration toxicity is not a relevant endpoint for metam.

MITC:
No data are available from humans. As MITC is a solid substance and no liquid hydrocarbon, aspiration toxicity is not a relevant endpoint for MITC.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Metam (incl. -sodium and -potassium):

No data are available from humans. As metam is a solid substance and no liquid hydrocarbon, aspiration toxicity is not a relevant endpoint for metam.

MITC:
No data are available from humans. As MITC is a solid substance and no liquid hydrocarbon, aspiration toxicity is not a relevant endpoint for MITC.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Metam (incl. -sodium and -potassium):
No data from animal studies or humans are available, which may be indicative of the potential of metam for aspiration hazard in humans. Furthermore, as metam is a solid substance no classification is warranted.

MITC:
No data from animal studies or humans are available, which may be indicative of the potential of MITC for aspiration hazard in humans. Furthermore, as MITC is a solid substance no classification is warranted.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Metam			
Acute oral toxicity study Please refer to Vol. 3, section B.6.2	640, 800, 1000 and 1600 mg/kg bw Single application (gavage)	Clinical signs: piloerection, hunched posture, abnormal gait, lethargy, decreased respiratory rate, ptosis and increased salivation were observed in all dose groups. Anticipated body weight gains were observed in the second week after treatment. Mortality from 800 mg/kg bw onwards. No macroscopic abnormalities. No microscopic examination performed. Critical effect at 640 mg/kg bw: reduced general health.	[redacted] (1991) [redacted] DRAR **B.6.2.1/01**
Acute oral toxicity study Please refer to Vol. 3, section B.6.2	200 and 2000 mg/kg bw Single application (gavage)	Clinical signs 200 mg/kg bw: lethargy on day 1 in ♀ rats, no preterminal death, no gross necropsy change. 2000 mg/kg bw: lethargy in one rat, lethargy and convulsions in other rat, another rat was lethargic and recumbent. All the 3 ♀ rats died.	[redacted], 2002 [redacted] DRAR: **B.6.2.1/02**
Acute oral toxicity study Please refer to Vol. 3, section B.6.2	65, 130, 195, 260 mg/kg bw	No mortality was observed. **260 mg/kg bw: ↑ALT/AST, centrilobular hepatocyte necrosis** 195 mg/kg bw: ↑AST, no microscopic findings 65, 130 mg/kg bw: no effect on liver enzymes, no microscopic findings	Thompson (2002) Toxicol. Sci. 70 (2002) 269-280 DRAR: **B.6.2.1/03**
Acute dermal toxicity Please refer to Vol. 3, section B.6.2	2000 mg/kg bw	Mortality: 1/5 ♀ rat (day 2). Autopsy revealed slight congestion in the subcutaneous region. Clinical signs: all rats were hypothermic on day 2 of the study. There were no other signs of systemic reaction to treatment.	[redacted] 1991 DRAR: **B.6.2.2/01**
Acute dermal toxicity Please refer to Vol. 3, section B.6.2	2000 mg/kg bw	Mortality: none Clinical signs: none	[redacted], 2002 [redacted] DRAR: **B.6.2.2/02**
Acute inhalation toxicity study Please refer to Vol. 3, section B.6.2	1.23, 2.43, 3.03 and 3.15 mg/L (aerosol) 4 hours (whole body)	No mortality at 1.23 mg/L LC_{50}: Males: 2.20 mg/L Females: 2.95 mg/L Combined: 2.54 mg/L Clinical signs: all dose groups: partially closed eyes or discharge from eyes, irregular respiration, gasping, exaggerated respiratory movements (observed during and after exposure) Reversible in 1.23 mg/L dose group (within 8-13 days) Reduced body weight, food or water consumption: all dose groups, dose dependent, males more affected Macroscopy: Congestion of lung and gas-filled stomach in decedents. No treatment-related findings in surviving rats. Microscopy: Inflammation/necrosis of bronchial epithelium and alveolar congestion/oedema in decedents Liver: Focal necrosis (sometimes centrilobular hepatocyte necrosis) was seen in : - 1 / 2 ♂ rats treated at 2.43 mg/L, and in: 1 / 4 ♂ and 3 / 4 ♀ rats treated at 3.15 mg/L Signs of inflammation in the lung at terminal sacrifice in all dose groups (dose related incidence and severity)	[redacted] (1992) [redacted] DRAR: **B.6.2.3/01**

Acute inhalation toxicity study Please refer to Vol. 3, section B.6.2	6.8 mg/L (aerosol) 4 hours, nose only	LC_{50} > 6.8 mg/L	[redacted] (1986) [redacted] DRAR: **B.6.2.3/02**
Acute inhalation toxicity study Please refer to Vol. 3, section B.6.2	2.98, 3.21 and 3.90 mg/L (aerosol) 4 h inhalation	Mortality 2.98 mg/L: 4/10, 3.21 mg/L: 7/10, 3.90 mg/L: 9/10 Clinical signs Salivation, lethargy, dyspnoea, tremors, ataxia, resorbed from day 3 (2.98 mg/L) to 6 (3.90 mg/L) onwards. Reduced bw.	[redacted] 2002 [redacted] DRAR: **B.6.2.3/03**
Acute Skin Irritation/ Corrosivity Please refer to Vol. 3, section B.6.2	0.5 mL = 266.5 mg (24H dermal application)	Skin necrosis. Metam is considered to be corrosive to the skin.	[redacted] .; 1991 [redacted] DRAR: **B.6.2.4/02**
Acute neurotoxicity study Please refer to Vol. 3, section B.6.7	50, 750, 1500 mg/kg bw Single application (gavage)	Reduced body weight changes from 750 mg/kg bw onwards. Macroscopy: Reddened: adrenals, lymph node, stomach, (red material around nose) No microscopic examination performed (except for neurotoxicity related information). Critical effect at 1210 mL/kg bw: reduced general health (sedation, comatose condition), mortality	[redacted] (1993) [redacted] DRAR: **B.6.7.1/01**
MITC			
Acute Oral Toxicity study Please refer to Vol. 3, section B.6.8.1.1	68.1, 100, 147, 215 mg/kg bw Single application (gavage)	**Target organ**: gastrointestinal tract/stomach (considered secondary to corrosive properties of MITC)	[redacted] (1986) [redacted] DRAR: **B.6.8.1.1/01**
Acute Dermal Toxicity Please refer to Vol. 3, section B.6.8.1.1	2000 mg/kg bw	**Target organ:** gastrointestinal tract/stomach (considered secondary to corrosive properties of MITC)	[redacted] (1987) [redacted] DRAR: **B.6.8.1.1/02**
Acute Inhalation Toxicity study Please refer to Vol. 3, section B.6.8.1.1	0.282, 0.496, 0.570, 0.628, 0.786, 1.64 mg/L (aerosol) 4 h inhalation (whole-body).	**Target organ:** respiratory tract (oedema, bronchiolitis, pneumonitis), lung (weight increase) (considered secondary to corrosive properties of MITC)	[redacted] (1981) [redacted] DRAR: **B.6.8.1.1/03**
Acute Skin Irritation/ Corrosivity Please refer to Vol. 3, section B.6.8.1.1	0.5 mL (24h dermal application)	**Target organ**: skin (necrosis) MITC is considered to be corrosive to the skin.	[redacted] (1986) [redacted] DRAR: **B.6.8.1.1/04**

Acute neurotoxicity study Please refer to Vol. 3, section B.6.8.1.6.	0, 20, 40, 80 ppm (corresponding to 0, 60, 120 and 240 mg/m³) Inhalation (whole body) 6 hours Vehicle: air	No mortality was observed in any dose group. 240 mg/m³ (66.42 mg/kg bw/d) microscopic findings in larynx, pharynx, transitional, respiratory and olfactory epithelial mucosae of nose. decreased locomotor activity 120 mg/m³ (34.02 mg/kg bw/d) microscopic findings in larynx, pharynx, transitional, respiratory and olfactory epithelial mucosae of nose. decreased locomotor activity 60 mg/m³ (16.2 mg/kg bw/d) effects on several FOB parameters NOAEC = < 20 ppm (60 mg/m³) for local or portal of entry effects NOAEC = 82 ppm (246 mg/m³) for acute neurotoxicity **Target organ: respiratory tract**	[redacted] (2011) [redacted] DRAR: **B.6.8.1.6.1**

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	**Test substance**	**Route of exposure Relevant information about the study (as applicable)**	**Observations**	**Reference**
Metam				
No data available				
MITC				
Case report	MITC	Acute MITC Poisoning caused by drinking 50 g of diluted MITC.	Retrosternal burning, epigastric pain and vomiting were the first signs of intoxication followed by deep coma with pulse 98/min and blood pressure 90/60 mm Hg. Further, a complete loss of all reflex and motor activity. Eight hours after the intoxication with MITC the patient died and necrotic mucosa was observed in the oesophagus, stomach, and proximal part of the duodenum.	[redacted] (1981) DRAR : **B.6.9.3/03**

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
METAM				
Sub-acute 8-day range-finding Study Please refer to Vol. 3, section B.6.3	Metam Batch n°: Y06930/007 Purity 525.54 g/L	Oral, drinking water Dose levels: 0, 0.35, 0.62 mg/mL 9-10/sex/group: young animals 15/sex/group: old animals	No mortality, no clinical signs or macroscopic findings were observed. 0.35, 0.62 mg/mL: ↓bw, ↓water consumption	[redacted] (1990) [redacted] DRAR: **B.6.3.1.1/01**
Sub-acute 21-day range-finding study Please refer to Vol. 3, section B.6.3	Metam Batch n°:11877-9-1 Purity 32.8 g/L	Oral, drinking water Dose levels: 2 days: 0, 1, 3, 6 mg/mL After a 5d recovery period, dosing was continued at: 0, 0.1, 0.3, 0.7 mg/mL 0, 10.5, 26.7, (NA) mg/kg bw/day 5/sex/group	Stains around the nose and thin appearance were observed. No mortality was observed. No significant effects on organ weights, except occasional ↑relative kidney weight and ↓absolute spleen weight. 0.1, 0.3, 0.7 mg/mL: ↓bw, ↓water consumption	[redacted] (1991) [redacted] DRAR: **B.6.3.1.2/01**
Developmental toxicity, rat Please refer to Vol. 3, section B.6.6	Metam sodium Batch n°: ZH130585 Purity 42.2%	Dose levels: 0, 10, 40, 120 mg/kg bw/day on gestation days 6-15	No macroscopic findings. No microscopy performed. No organ specific effects observed **NOAEL (maternal)**: 10 mg/kg bw/d **NOAEL (developmental):** <10 mg/kg bw/d **Critical effects at the LOAEL**: Reduced bw and fc (maternal toxicity). Increased incidence of a number of variations and retardations as well as reduced foetal and placenta weight.	[redacted] [redacted] (1986) [redacted] DRAR: **B.6.6.2.1/01**
Developmental toxicity, rat Please refer to Vol. 3, section B.6.6	Metam sodium Batch n°: BAS/005/00N Purity 43.1%	Dose levels: 0, 5, 20, 60 mg/kg bw/day on gestation days 7-16	Macroscopic findings: pelvic dilatation (4/24 animals at 60 mg/kg bw/d); severity: slight to moderate (either left or right kidney). No microscopy performed. No microscopy performed. No other organ specific effects observed. **NOAEL (maternal)**: 5 mg/kg bw/d **NOAEL (developmental):** <5 mg/kg bw/d **Critical effects at the LOAEL**: Maternal: ↓bw and fc Developmental: ↑incidence of a number of variations, ↓fœtal weights.	[redacted] [redacted] (1993) [redacted] DRAR: **B.6.6.2.1/03**
Developmental toxicity, rabbit Please refer to Vol. 3, section B.6.6	Metam sodium Batch n°: ZH 130585 Purity 42.2%	Dose levels: 0, 10, 30, 100 mg/kg bw/day on gestation days 6-18	No treatment-related macroscopic findings No microscopy performed. No organ specific effects observed **NOAEL (maternal)**: 10 mg/kg bw/d **NOAEL (developmental)**: <10 mg/kg bw/d **Critical effects at the LOAEL:** Maternal: ↓bw, bw gain and fc Developmental: ↑incidence of dead implantations and ↓live foetuses.	[redacted] [redacted] (1987) [redacted] DRAR: **B.6.6.2.2/04**
Developmental toxicity, rabbit Please refer to Vol. 3, section B.6.6	Metam sodium Batch n°: 90/2 Purity 43.14%	Dose levels: 0, 5, 20, 60 mg/kg bw/day on gestation days 8-20	No treatment-related macroscopic findings No microscopy performed No organ specific effects observed **NOAEL (maternal):** 5 mg/kg bw/d **NOAEL (developmental):** 20 mg/kg bw/d **Critical effects at the LOAEL**: Maternal: ↓bw and fc Developmental: ↑foetotoxicity.	[redacted] (1993) [redacted] DRAR: **B.6.6.2.2/03**

MITC				
Sub-acute 28-day study Please refer to Vol. 3, section B.6.8.1.2	MITC Batch n°: 6205 MK Purity 96.9%	Inhalation, whole body Dose levels: 0, 5, 20, 100 mg/m³ 4-week period for 6h/d (5 d/wk; in total: 20 exposures) Vehicle = filtered air	**Target organ**: respiratory tract (oedema, bronchiolitis, pneumonitis), lung (↑weight) (considered secondary to corrosive properties of MITC)	[redacted] (1987) [redacted] DRAR: **B.6.8.1.2.1/01**
Sub-acute 28-day study Please refer to Vol. 3, section B.6.8.1.2	MITC Batch n°: 56198PJV Purity 99.7%	Inhalation, whole body Dose levels: 0, 5, 20, 40 and 80 ppm (corresponding to 0, 15, 60, 120, 240 mg/m³) 4-week period for 6h/d (5 d/wk; in total: 20 exposures) Vehicle = filtered air	**Target organ:** respiratory tract (oedema, bronchiolitis, pneumonitis), ↑lung weight (considered secondary to corrosive properties of MITC). ↓Thymus weight	[redacted] (2011) [redacted] DRAR: **B.6.8.1.2.1/02**
Sub-acute 28-day study Please refer to Vol. 3, section B.6.8.1.2	MITC Batch n°: 56198PJV Purity 99.7%	Inhalation, whole body Dose levels: 0, 5, 20, 40, 80 ppm (~ 0, 15, 60, 120, 240 mg/m³) 4-week period for 6h/d (5 d/wk; in total: 20 exposures) Vehicle = filtered air	**Target organ:** Respiratory tract (oedema, bronchiolitis, pneumonitis) (considered secondary to corrosive properties of MITC)	[redacted] (2013) [redacted] DRAR: **B.6.8.1.2.1/03**
Developmental toxicity study in rats Please refer to Vol. 3, section B.6.8.1.5	MITC Batch n°: 56198PJV Purity 99.7%	Inhalation, whole body Dose levels: 0, 1, 4, 12 ppm (~ 0, 3, 12, 36 mg/m³)	**No target organ identified** **12 ppm** Dams: ↓Body weight (change), food consumption Pups: ↑occurrence of major blood vessel variation and reduced ossification of the 13th rib **1 and 4 ppm** Dams: No treatment-related effects Pups: No treatment-related effects **NOAEL (general toxicity): 4 ppm**	[redacted] (2012) [redacted] DRAR: **B.6.8.1.5.2/03**
Developmental toxicity study in rabbits Please refer to Vol. 3, section B.6.8.1.5	MITC Batch n°: 56198PJV Purity 99.7%	Inhalation, whole body Dose levels: 0, 1, 5, 15 ppm (equivalent to 0, 3, 15, 45 mg/m³)	**No target organ identified** **15 ppm** Dams: ↑**mortality**, eye closure, ↑respiration, ↓body weight (change), food consumption Pups: Single developmental findings **1 and 5 ppm** Dams:No treatment-related effect Pups:No treatment-related effects **NOAEL (general toxicity): 5 ppm, NOAEL (developmental): 5 ppm**	[redacted] (2012) [redacted] DRAR: **B.6.8.1.5.2/06**

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Metam (incl. -sodium and -potassium):

In acute toxicity studies effects beside lethality and general poor condition were observed on the liver (oral, inhalation) and respiratory tract (inhalation). Clinical signs included partially closed eyes or discharge from eyes, irregular respiration, gasping, exaggerated respiratory movements (observed during and after exposure). Inflammation/necrosis of bronchial epithelium and alveolar congestion/oedema in decedents as well as focal necrosis in the liver was observed in decedents. In short term oral or inhalation range finding studies in rats or mice the most critical effect was body weight loss and reduction of water consumption. In developmental toxicity studies in rats and rabbits, similar effects were observed on body weight and water consumption as in the subacute studies. No target organ specific findings were observed.

Furthermore, the results of the skin irritation/corrosion study were used for further assessment. Metam was shown to be corrosive in the skin irritation/corrosion study.

MITC:

For the assessment on STOT SE classification acute oral, dermal, inhalation and neurotoxicity studies in rat and mouse were taken into consideration. Furthermore, the results of the skin irritation/corrosion study were used for further assessment.

MITC was shown to be corrosive in the skin irritation/corrosion study.

In the combined acute inhalation toxicity/neurotoxicity effects on the lung, eyes and respiratory tract were observed. No effects on neuropathologic parameters were observed. Effects on FOB parameters were observed and are discussed in "Section 2.6.7 Neurotoxicity". They are not considered relevant for STOT SE 1/2 classification and no indication of narcotic effects were observed. This is in concordance with the effects observed in the acute and subacute inhalation studies were the respiratory tract and the lung were the target organs. In the acute inhalation toxicity study additional effects in the liver (focal necrosis) was observed in the decedents. In the animals sacrificed at terminal sacrifice occasional foci of mononuclear cells was observed in a low incidence uniformly distributed in control and treated groups. The effect on the liver was considered secondary of the corrosive/general toxic properties of MITC. In the acute oral and dermal toxicity studies the gastrointestinal tract/stomach has been the target organ, which was also considered to be due to the corrosive properties of MITC.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

According to the ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling, and packaging (CLP) of substances and mixtures, version 5.0 of July 2017, substances are to be classified for STOT SE if they cause specific, non-lethal target organ toxicities resulting from single exposures to the substance. In cases where a single exposure to a substance causes lethality, that effect should result in classification for acute toxicity, not for STOT SE.

Classification into STOT SE, Category 1 or 2 might be appropriate, if a substance is presumed to produce significant and/or severe target organ toxicity in humans following single exposure, on the basis of observations in humans or evidence from animal studies or is presumed to have the potential to cause harm to human health following single exposure.

If a study shows clear evidence for narcotic effects or respiratory tract irritation at any dose level then this could support classification with Category 3. Classification for corrosivity is considered to implicitly cover the potential to cause respiratory tract irritation and so the additional Category 3 is considered to be superfluous.

Metam (incl. -sodium and -potassium):

The effects observed on the respiratory tract with metam-sodium after single or short-term exposures could be related to the corrosive properties of metam. These effects are considered to be covered by the classification of metam as "corrosive". Therefore, neither classification in categories 1/2 or 3 (respiratory tract irritation) is proposed.

Effects on the **liver** (focal necrosis) were observed in rats of the acute inhalation toxicity study at dose levels related to mortality ([redacted], 1992).

The liver histopathology of rats which died during the test revealed focal necrosis (sometimes centrilobular hepatocyte necrosis) in 1/4 ♂ and 3/4 ♀ rats treated with 3.15 mg/L metam sodium, and 1/2 ♂ rats treated with 2.43 mg/L metam sodium. Among the rats killed following the 14-day observation period, no liver necrosis was observed at any dose.

These effects are noted at the doses of **2.43 and 3.15 mg/L**/4h , which are higher than the guidance value of STOT-SE Cat.1, (*i.e.* inhalation (rat) (aerosol) C ≤ 1.0 mg/L/4h), but within the guidance values for Cat.2 (1.0 < C ≤5.0 mg/L/4h), but yet higher than the LC_{50} = 2.54 mg/L corresponding to an acute toxicity estimate (ATE) in the range of 0.5<ATE≤2.0 mg/L.

However, in the publication (evaluated in Vol.3, B.6.2.1/03) from Thompson (2002) single oral exposure of metam at dose levels not leading to mortality, induced necrosis in the liver. Effects on the liver after single exposure were seen in 3/3 treated rats at 195-260 mg/kg bw *i.e.* a dose which is < LD_{50} (870 mg/kg b.w., in ♂).

According to the CLP guidance document effect levels <300 mg/kg bw after oral exposure in rats would trigger classification as STOT SE, Category 1. Therefore, metam is proposed to be classified as **STOT SE, Category 1 based on damage of the liver**.

MITC:

The effects observed with MITC after single or short-term exposures could be related to the corrosive properties of MITC. The target organs were the **respiratory tract** (after inhalation exposure) or gastrointestinal tract (after oral/dermal exposure). These effects might be considered to be covered by the classification of MITC as "corrosive". However, taking into account the severity of these effects on respiratory tract, a classification as specific target organ toxicity – single exposure 3 (**STOT-SE 3 – H355 may cause respiratory irritation**) could formally be considered. However, since available data indicate that the mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of

Table 3.1.3 in Annex I, the sentence **EUH071 –'Corrosive to the respiratory tract'** is proposed, and takes precedence on H355.

Effects on the liver (focal necrosis) were only observed in rats of the acute inhalation study dying before the end of the study. Therefore, these effects are considered to be covered by the classification for acute toxicity taking into account lethality.
Effects on the liver (focal necrosis) were observed in rats of the acute inhalation toxicity study at dose levels related to mortality ([redacted], 1981, see DAR Vol.3, B.6.8.1.1/03). The liver histopathology of rats which died during the test revealed focal necrosis (sometimes centrilobular hepatocyte necrosis, and sometimes with acute inflammatory cell infiltration) in 1/1♂ and 1/1♀ rats treated with 0.496 mg/L MITC, 1/3♂ and 0/3♀ rats treated with 0.57 mg/L MITC, and 3/5♂ and 2/5♀ rats treated with 0.628 mg/L MITC. Among the rats killed following the 14-day observation period, no liver necrosis was observed at any dose.

These effects are noted at the doses between **0.496 and 0.628 mg/L**/4h , which are lower than the guidance value of STOT-SE Cat.1, (*i.e.* inhalation (rat) (aerosol) C ≤ 1.0 mg/L/4h, but yet higher than the LC_{50} = 0.54 mg/L corresponding to an acute toxicity estimate (ATE) in the range of 0.5<ATE≤2.0 mg/L.

In the Guidance, it is stated that care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at doses relevant for classification for acute toxicity, the latter will take precedence and STOT-SE will not be assigned. This situation seems to be the case for MITC.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Metam (incl. -sodium and -potassium):
STOT SE, Category 1 (H370: Causes damage to liver)

MITC:
EUH071 –'Corrosive to the respiratory tract'

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Metam			
Mouse			
Subchronic 90-day mouse study Similar to OECD TG 408 (2018) Restricted stability analysis prevents accurate actual substance intake Clinical biochemistry not performed Haematology: no blood clotting determined Organ weights not determined; epididymides, heart, prostate and seminal vesicles with coagulating glands as a whole, ovaries, uterus, thymus, pituitary gland and spleen. Vagina not collected Mammary gland: females only Oestrus cycle not determined GLP compliant (no attest of competent authority) Mouse, C57BL/10jfAP/Alpk 90-d: 10/sex/group 28-d sacrifice: 28/sex/group	Metam sodium Batch n°: BAS/005/00N Purity 52.6% Oral, drinking water Dose levels: 0, 0.018, 0.088, 0.35, 0.62 mg/mL Actual intake (mg/kg bw/d): 0.79, 4.48, 36, 60 (males) 1.05, 5.82, 38, 64 (females) 90-days, daily (7 days/week) Vehicle = drinking water Oral, drinking water	<u>0.62 mg/mL</u> Anaemia ↓bw (~10%), ↓bw gain (10-20%), ↓fc(5-20%), ↓wc ↑liver weight (20-30%), interim/terminal sacrifice ↑kidney weight (10%), terminal sacrifice Bladder: mucosal hyperplasia, cystitis, eosinophilic granules in epithelium <u>0.35 mg/mL</u> Anaemia ↓bw (~10%), ↓bw gain (26%, males), ↓fc (5-10%), ↓wc ↑liver weight (20-30%), interim/terminal sacrifice ↑kidney weight (10%), terminal sacrifice Bladder: mucosal hyperplasia, cystitis, eosinophilic granules in epithelium <u>0.088 mg/mL</u> Anaemia ↓wc ↑liver weight (5-10%), terminal sacrifice Bladder: eosinophilic granules in epithelium <u>0.018 mg/mL</u> No adverse effect **NOAEL =** 0.018 mg/ml = 0.79 mg/kg bw/d. **Critical effects at the LOAEL** = 0.088 mg/ml = 4.48 bw/d: ↓water consumption, haematological changes (anaemia), histopathological changes in bladder (eosinophilic granules in epithelium)	[redacted] (1991) [redacted] DRAR: **B.6.3.2.1/01**
Rat			

Subchronic 90-day rat study US EPA 82-1 Restricted stability analysis prevents accurate actual substance intake. Clinical biochemistry: HDL, LDL, blood urea nitrogen, total T4, T3 and TSH not measured. Organ weights not determined: epididymides, heart, prostate and seminal vesicles with coagulating glands as a whole, ovaries, uterus, thymus, pituitary gland and spleen. Cervix, vagina and coagulation glands were not collected and histopathologically examined. Spinal cord, bone, eyes, skin, mammary gland and muscle were collected but not histopathologically examined. Brain histopathology is limited Oestrus cycle was not determined GLP compliant (no attest of competent authority) Rat, Alpk:APfSD 12/sex/group	Metam sodium Batch n°: BAS/005/00N Purity 52.6% Oral, drinking water Dose levels: 0, 0.018, 0.089, 0.443 mg/mL Actual intake (mg/kg bw/d) (California EPA, 2004): 0.49, 3.1, 18.51 (males) 0.73, 3.68, 21.05 (females) 90-days, daily (7 days/week) Vehicle = drinking water	<u>0.443 mg/mL</u> Anaemia (slight) Clinical chemistry ↓bw (~20%), ↓fc (20-40%), ↓wc (47-72%) ↑kidney/liver weight Kidney: tubular basophilia Nasal cavity, olfactory epithelium: disorganisation, vacuolated Bowman glands, vacuolation <u>0.089 mg/mL</u> Anaemia Clinical chemistry ↓bw (~5%, females), ↓fc (6%,females), ↓wc (32%, females) ↑ liver weight <u>0.018 mg/mL</u> Anaemia (slight, females) No adverse effects **NOAEL** = 0.018 mg/mL = 0.49/0.73 mg/kg bw/d) **Critical effects at the LOAEL** = 0.089 mg/mL = 3.1/3.7 mg/kg bw/d: ↓body weight and ↓body weight gain resulting from ↓water consumption (supported by ↓urinary volume and ↑specific gravity) altering food consumption.	[redacted] (1991) [redacted] DRAR: **B.6.3.2.2/01**

Subchronic 90-day study Equivalent to OECD 413 (1981) Blood analysis not performed in all animals. Study was performed with a formulation. GLP compliant (no attest of competent authority Rat SD 12/sex/group 6/sex/group for hematology	Metam (Vapam technical) Batch n°: EHC #0355-21 Purity 32.7% Inhalation (whole body) Dose levels (actual conc.): 0, 6.5, 45, 160 mg/m³ 0, 1.75, 12.1, 42.6 mg/kg bw/d (MITC concentrations: 0.189, 0.59, 1.54 mg/kg bw/d) 65-day period for 6 hours per day (5 days/week) Vehicle = filtered air	<u>160 mg/m³ (42.6 mg/kg bw/d)</u> 1 male died (renal failure) ↓bw (6-11%, males; 10-14%, females) ↓bw gain ↓fc Clinical chemistry Lung/stomach: coloured foci Skin: brown staining (females) Relative organ weight and respective histopathology (no statistically significant changes): Lung (histiocytosis) Liver (no histopathological correlates) Kidney (pyelitis) Histopathology: Stomach (gastritis; ectasia, glandular duct) Urinary tract (hyperplasia/metaplasia; 1/18 females) Nasal passage: rhinitis, hyperplasia of mucogenic epithelium (very slight) <u>45 mg/m³ (12.1 mg/kg bw/d)</u> ↓bw gain ↓fc Clinical chemistry Skin: brown staining (females) Relative organ weight and respective histopathology (no statistically significant changes): Liver (no histopathological correlates) Histopathology: Nasal passage: rhinitis, hyperplasia of mucogenic epithelium (very slight) Stomach (ectasia, glandular duct) <u>6.5 mg/m³ (1.75 mg/kg bw/d)</u> Except for LDH, total protein and globulin alpha-2 no statistically significant changes were observed. ↓bw gain ↓fc Clinical chemistry Lung: coloured foci Skin: brown staining (females) Histopathology: Nasal passage: rhinitis, hyperplasia of mucogenic epithelium (very slight) Stomach (ectasia, glandular duct) **Local NOAEL** = 1.75 mg/kg bw/d **Systemic NOAEL** = 1.75 mg/kg bw/d **Critical effects at the LOAEL**: **Local** (12.1 mg/kg bw/d): histopathology: nasal passage inflammation (lymphocytic rhinitis, hyperplasia of mucogenic epithelium). **Systemic** (12.1 mg/kg bw/d): ↓body weight gain ↓food consumption, serum chemical changes, some of which may indicate liver damage and nasal stress revealed by histopathology.	[redacted] (1983) [redacted] DRAR: **B.6.3.4.1/01** Re-examination of liver sections: [redacted] [redacted] (1992) [redacted] DRAR: **B.6.3.4.1/01**

Subchronic neurotoxicity 90-day rat study Equivalent to OECD TG 424 (1997) Animal observations was limited to 1 day, FOBS were not performed before 5-week exposure (recommendation: during week 1-2) Histopathology was performed only for control and top doses. GLP compliant (no attest of competent authority) Rat,Alpk:APfSD 12/sex/group	Metam sodium Batch n°: BAS/005/00n 90-2 Purity 43.15% Oral, drinking water Dose levels: 0, 0.02, 0.06, 0.2 mg/mL (0, 2, 6, 14.7 (m) and 0, 3.3, 8.4, 17.8 (f) mg/kg bw/d) 90-days, daily (7 days/week) Vehicle: drinking water drinking water	All dose groups: No effects on brain weight, length or width. No histopathological effects observed. <u>0.2 mg/mL</u> ↓bw (~10%) ↓fc, ↓wc ↓Landing foot splay (wk 9, males) <u>0.06 mg/mL</u> ↓bw (<10%, females) ↓fc, ↓wc <u>0.02 mg/mL</u> ↓bw (<10%, females) ↓fc, ↓wc **NOAEL** = 0.2 mg/mL (14.7/17.8 mg/kg bw/d) **Critical effects at the LOAEL** (>14.7 mg/kg bw/d): not applicable	[redacted] (1994) [redacted] DRAR: **B.6.7.1/03**
Dog			
Subchronic 90-day dog study US EPA 82-1 Not fully in agreement with Dir 87/302 or OECD TG 409 (1998-1981) The highest dose level induced death and was, therefore, chosen too high. Clinical biochemistry parameters did not include ornithine decarboxylase. Urine was collected for urinalysis in the week prior to termination only instead of at the start, midway and at the end of the study. The following organs were not weighed: gall bladder, ovaries, uterus, thymus and spleen. GLP compliant (no attest of competent authority) Dog, Beagle 4/sex/group	Metam sodium Batch n°: BAS/005/00N Purity 43.15% Oral, gelatine capsules Dose levels: 0, 1, 5, 10 mg/kg bw/d 90-days, daily (7 days/week)	<u>10 mg/kg bw/d</u> Mortality: 1 male (wk 12) and 1 female (wk 11) Clinical signs Clinical chemistry and haematology (several parameters, dose-related) ↓bw (5-14%, males) ↑kidney weight (males) Liver: hepatitis Bladder: increased mitosis (3/3 males, 2/3 females) <u>5 mg/kg bw/d</u> Clinical signs Clinical chemistry and haematology (several parameters, dose-related) ↓bw (5-14%, males) ↑kidney weight (males) Liver: hepatitis Bladder: increased mitosis (1/4 males) <u>1 mg/kg bw/d</u> Haematology: ↑Lymphocyte count (35%) Clinical chemistry: ↑ALT (371%, females) **NOAEL** = 1 mg/kg bw/d **Critical effects at the LOAEL** (5 mg/kg bw/d): increased urinary bladder cell mitosis, liver hepatitis and clinical signs of toxicity (salivation, food regurgitation)	[redacted] (1992) [redacted] DRAR: **B.6.3.2.3/01**

1-year dog study US EPA 83-1 Not fully in agreement with Dir 87/302/EEC Annex V or OECD TG 452 (1981) The dose levels were chosen too low; the highest dose level did not clearly elicit evidence of toxicity and no clear dose-response-relationship was evident. The following organs were not weighed: gall bladder, heart, ovaries, uterus and spleen. Coagulating gland, Harderian gland, lacrimal gland and vagina were not collected and histopathologically examined. Nevertheless, these deviations are considered not to affect the study outcome and thus, the current study is considered acceptable. GLP compliant (no attest of competent authority) Dog, Beagle 4/sex/group	Metam sodium Batch n°: BAS/005/00N 90-2 Purity 43.15% Oral, gelatine capsules Dose levels: 0, 0.05, 0.1, 1 mg/kg bw/d 1-year, daily (7 days/week)	<u>1 mg/kg bw/d</u> Clinical chemistry and haematology (↑Kaolin-cephalin time, ↑ALT/ASP/ALP) ↓bw (4 in males-8% in females at wk 53) Liver: hepatocyte/macrophage/Kupffer cell pigmentation <u>0.1 mg/kg bw/d</u> ↓bw (8%, females) <u>0.05 mg/kg bw/d</u> No adverse effects **NOAEL = 0.1 mg/kg bw/d)** **Critical effects at the LOAEL** (1 mg/kg bw/d): effects suggesting hepatotoxicity, blood chemistry effects (↑kaolin-cephalin time, ↑ALT/ASP/ALP), and associated liver histopathological findings (hepatocyte and Kupffer-cell pigmentation).	[redacted]. (1994) [redacted] DRAR: **B.6.3.3/01**
Subchronic 90-day dog study No guideline followed Similar to OECD TG 409 (1998-1981): Focus of investigation was on liver injury Not GLP compliant Dog, Beagle 1/sex/group	Metam sodium Batch n°: BAS/005/00N Purity 43.14% Oral, gelatine capsules Dose levels: 10 mg/kg bw/d Dosing for 12 (f) and 13 (m) weeks. Recovery was then monitored for 8 weeks.	Effects at 10 mg/kg bw/d: Sustained elevations of plasma ALT in weeks 10-12 (f) and 11-13 (m) No macroscopic findings of effects on liver weight Slight increase in number of pigmented macrophages/Kuppfer cells Liver effects of metam considered completely reversible.	[redacted]. (1993) [redacted] DRAR: **B.6.3.2.3/02**
Rabbit			

Subacute 21-day rabbit study Equivalent to OECD TG 410 (1981) Dose levels considered too low for evaluation of systemic toxicity. Not GLP compliant (not mandatory at that time) Rabbit White Russian 10/sex/group	Metam sodium Batch n°: BAS-00500-N Purity 42.4% Dermal, 8-h/day Dose levels: 31.25, 62.5, 125 mg/kg bw/day Vehicle: 0.8% aqueous hydroxypropyl-methyl-cellulose gel (type E4M) Recovery group: 2/sex/group were observed for additional 21 days after exposure	125 mg/kg bw/d Erythema/Edema Dermatitis (marked) (reversible) No systemic effects observed 62.5 mg/kg bw/d Erythema/Edema Dermatitis (slight) (reversible) No systemic effects observed 31.25 mg/kg bw/d No local or systemic effects observed **Local NOAEL** = 31.25 mg/kg bw/d based on erythema/dermatitis **Systemic NOAEL** = 125 mg/kg bw/d **Critical effects at the LOAEL**: **Local** (62.5 mg/kg bw/d), erythema/dermatitis **Systemic** (>125 mg/kg bw/d): not applicable.	[redacted]. (1979) [redacted] DRAR: **B.6.3.4.2/01**
MITC			
Rat			
Subacute 28-day rat study OECD TG 412 (2018) Stability (expiry date), air changes per hour and acclimation period GLP compliant Rat, Wister 5/sex/group	MITC Batch n°: 6205 MK Purity 96.9% Inhalation (whole body) Dose levels: 0, 5, 20 and 100 mg/m³ (corresponding to 0, 1.35, 5.4, 27.0 mg/kg b.w./d) 4-week period for 6 hours per day (5 days/week; up to a total of 20 exposures) Vehicle = filtered air	No deaths occurred during the study period. 100 mg/m³ mucosal and respiratory irritation led to changes in the breathing patterns and deterioration of the general state of health; not reversible clinical chemistry, haematology and organ weights (lung) affected 20 mg/m³ Mucosal and respiratory irritation led to changes in the breathing patterns and deterioration of the general state of health; reversible 5 mg/m³ No testsubstance-related systemic findings. Focal atrophy in olfactory epithelium. **Local NOAEL** = <5 mg/m³ = <1.35 mg/kg bw/d **Systemic NOAEL** = 5 mg/m³ = 1.35 mg/kg bw.d **Critical effects at LOAEC**: **Local:** ↑focal atrophy in olfactory epithelium at 5 mg/m³ **Systemic**: ↓body weight (gain), ↑clinical signs, ↑non-focal atrophy olfactory epithelium, ↑neutrophils) at 20 mg/m³	[redacted] (1987) [redacted] DRAR: **B.6.8.1.2.1/01**

Sub-acute 28-day rat study OECD TG 412 (2018) Broncho-alveolar lavage and ophtalmological examination not performed GLP compliant Rat, Crl:CD (Sprague Dawley) 5/dsex/group	MITC Batch n° 56198PJV (Purity 99.7%) Inhalation (whole body) Dose levels: 0, 5, 20, 40 and 80 ppm (corresponding to 0, 15, 60, 119, 235 mg/m^3) (corresponding to 0, 4.21, 16.15, 32.05, 63.21 mg/kg b.w./d) 4-week period for 6 hours per day (5 days/week; up to a total of 20 exposures) Vehicle = filtered air	Due to overt toxicity, the last exposure for the 80 ppm group males and females was study day 14 and 15, respectively. On study day 16, all surviving 80 ppm group males and females were subjected to an early termination. Test substance-related deaths were noted in the 80 ppm group. 1 male and 1 female rat were found dead on study days 9 and 15, respectively, and 1 male and 1 female were euthanised in extremis on study days 12 and 15, respectively. <u>80 ppm (= 235 mg/m³)</u> ↑ mortality ↓ BW and overall BWG ↓ food consumption microscopic findings in the respiratory tract <u>40 ppm (= 119 mg/m³)</u> ↓ BW and overall BWG; microscopic findings in the respiratory tract <u>20 ppm (= 60 mg/m³)</u> ↓ BW and overall BWG; ↑histopathological alterations in the respiratory tract. <u>5 ppm (= 15 mg/m³)</u> ↑clinical signs, ↓body weight gain (>10%), ↓thymus weight. No test substance-related findings in the nasal cavity In all affected regions of the respiratory tract, epithelial regeneration was present suggesting ongoing resolution of lesions and possible reversibility. **Local NOAEL** = 5 ppm (= 15 mg/m^3 = 4.21 mg/kg bw/d) **Systemic NOAEL** = <5ppm (= <15 mg/m^3 = <4.21 mg/kg bw/d) **Critical effects at LOAEL**: **Local:** effects in the respiratory tract, e.g. single cell necrosis and regeneration of epithelial cells (lung, larynx) at ≥ 20 ppm **Systemic:** ↑clinical signs, ↓body weight gain (>10%), ↓thymus weight at 5 ppm.	[redacted] (2011) [redacted] DRAR: **B.6.8.1.2.1/02**

Sub-chronic 90-day rat study OECD TG 413 (2018) Broncho-alveolar lavage and ophthalmological examination not performed GLP compliant Rat, Crl:CD (Sprague Dawley) 10/sex/group	**MITC** Batch n°: 56198PJV Purity 99.7% Inhalation (whole body) Dose levels: 0, 1, 5 and 15 ppm (corresponding to 0, 3, 15 and 45 mg/m³) (corresponding to 0, 0.81, 4.0, 12 mg/kg b.w./d) 13-week period for 6 hours per day (5 days/week; up to a total of 65 exposures) Vehicle = filtered air	No MITC-related effects on survival, clinical observations or food consumption. <u>15 ppm (= 45 mg/m^3 = 12 mg/kg bw/d)</u> histopathological changes in the upper respiratory tract (URT; nasal cavity and larynx); respiratory tract lesions, which were diminished in severity with progression deeper into the airways <u>5 ppm (= 15 mg/m^3 = 4 mg/kg bw/d)</u> Epithelial findings, rarely; acute inflammation with nearby epithelial changes <u>1 ppm (= 3 mg/m^3 = 0.81 mg/kg bw/d)</u> epithelial findings were rarely **Local NOAEL** = 1 ppm (= 3 mg/m^3 = 0.81 mg/kg bw/d) for portal of entry effects in the upper respiratory tract **Systemic NOAEL** = 5 ppm (= 15 mg/m^3 = 4 mg/kg bw/d) for systemic effects **Critical effects at LOAEC**: **Local (upper respiratory tract)**: Inflammatory, regenerative and necrotic (single cell) changes at 15 mg/m³ (larynx, nasal level). **Systemic and local (lower respiratory tract)**: effects on hematology (neutrophils) and coagulation (APTT) parameters, ↑liver weight, ↓thymus weight at 45 mg/m³ in females	[redacted] (2012) [redacted] DRAR: **B.6.8.1.2.2/02**

Chronic 2-year rat study (12-month time point) OECD TG 453 (2018) only 5 animals/ sex for the chronic phase and recovery animals; haematological, clinical, biochemistry and urinalysis parameters were examined for the control and high dose group only; Clinical biochemistry parameters did not include haematocrit, creatinine, sodium and potassium examination; only one test for hepatobiliary evaluation; cervix, coagulating gland, lacrimal gland, parathyroid, rectum, skeletal muscle, vagina and bone marrow not collected and examined; only one level of the spinal cord collected and examined, epididymides and uterus not weighed, thyroids weighed without parathyroids Non GLP compliant Rat, Crl:CD (Sprague Dawley) 65/sex/group (5/sex/group for chronic and 60/sex/group for carcinogenicity phase)	MITC Batch n°:28 166; 29 482 Purity 97.2 - 99.7% Oral (drinking water) Dose levels: 0, 2, 10 and 50 ppm (corresponded to 0.08, 0.37 and 1.60 mg/kg bw/day for males and 0.12, 0.56 and 2.65 mg/kg bw/day for females) 52-week chronic phase and 104-week period Vehicle =untreated drinking water	No MITC-related deaths <u>50 ppm (= 1.60 and 2.65 mg/kg bw/day)</u> ↓ BW ↓ water intake Slight alterations of WBC, histopathology findings (bone marrow hyperplasia, increased kidney microcalculi, liver effects, spleen hyperplasia/increased haematopoiesis) <u>10 ppm (= 0.37 and 0.56 mg/kg bw/day)</u> No test substance-related findings <u>2 ppm (= 0.08 and 0.12 mg/kg bw/day)</u> No test substance-related findings **NOAEL** = 10 ppm corresponding to 0.44 and 0.66 mg/kg bw/day in males and females, respectively **Critical effects at LOAEL**: **Systemic**: Reduced BW (9%), WBC alterations, histopathological findings slightly increased	[redacted] (1984) [redacted] DRAR: **B.6.8.1.4.1/01**

Chronic 2-year study (12-month time point) OECD TG 453 (2018) No justification is provided regarding exposure for 5 days per week. GLP compliant Rat, Crl:CD (Sprague Dawley) 60/sex/group (10/sex/group for chronic phase and 50/sex/group for carcinogenicity phase)	MITC Batch n°: 56198PJV (Purity 97.2-99.7%) Inhalation (whole body) Dose levels: 0, 0.5, 5 and 20 ppm (corresponding to 0, 1.5, 15 and 60 mg/m³) (corresponding to 0, 0.65, 6.5, 26 mg/kg b.w./d) 52-week (chronic phase) and 104-week period for 6 hours per day with 5 days/week (exception: 4 days in week 25) Vehicle = filtered air	MITC-related deaths in the 20 ppm group males and females (related to nasal tumours or necrotizing lung lesions) <u>20 ppm (= 60 mg/m³)</u> ↓ BW and overall BWG ↓ food consumption rales, laboured respiration, clear and/or red nasal discharge and red material around the nose (males only); palpable masses on or near the nose; ↓ reticulocytes ↓ red blood cell distribution width ↓ glucose ↑ urea nitrogen ↑ leukocyte parameter (females) ↑ lung weight Microscopic eye findings of mild acute inflammation correlated with ophthalmic findings of bilateral keratitis; only treatment-related microscopic findings outside of the respiratory tract were in the olfactory bulb (brain) and eyes. <u>5 ppm (= 15 mg/m³)</u> squamous metaplasia and olfactory epithelial degeneration in nasal tissues and epithelial hyperplasia and squamous metaplasia in the larynx (males only) <u>0.5 ppm (= 1.5 mg/m³)</u> ↑squamous metaplasia , ↑olfactory nasal epithelium degeneration, ↑epithelial hyperplasia, ↑squamous metaplasia on the larynx **Local NOAEL = <0.5 ppm (< 1.5 mg/m³ ,<0.65 mg/kg bw/d)** **Systemic NOAEL = 5 ppm (=15 mg/m³ =0.65 mg/kg bw/d)** **Carcinogenic NOAEL =5 ppm (=15 mg/m³ =6.5 mg/kg bw/d)** **Critical effects at LOAEL**: **Local** (0.5 ppm): ↑squamous metaplasia, ↑olfactory nasal epithelium degeneration, ↑epithelial hyperplasia, ↑squamous metaplasia on the larynx at 0.5 ppm **Systemic** (20 ppm): No ↑mortality vs. control incidence, but at top-dose death caused by nasal tumours. Other relevant effects: ↓body weight (>20%), ↓body weight gain, ↑haematology/clinical chemistry findings at 20 ppm **Carcinogenic** (20 ppm): ↑malignant and benign nasal tumours, 1 benign papilloma in the lung at 20 ppm.	[redacted] (2015) [redacted] DRAR: **B.6.8.1.4.1/02**
Mechanistic study 1-day, 1-week, 4-week and 4-week/4-week recovery No guideline followed GLP compliant Rat, Crl:CD(SD) 8 males/group	MITC Batch n°: STBH5869 Purity 99.4% Inhalation (whole body) Dose levels: 0, 0.5, 5 and 20 ppm 6 hours per day (5 days/week) Vehicle = filtered air	No MITC-related effects on survival. Effects on food consumption, body weight and body weight gain at 20 ppm. No treatment-related changes of haematology or clinical chemistry parameters. The main findings of this study in rats were: 1) acute and subacute inhalation exposures to 5 and 20 ppm MITC caused dose-dependent effects including nasal histopathology and increased DNA synthesis/cellular replication in nasal epithelium, 2) no nasal histopathology or increased epithelial DNA synthesis/cellular replication were present in rats exposed to 0.5 ppm 3) squamous metaplasia of TE and RE was a common finding in 20 ppm, but not 0.5 or 5 ppm-, exposed rats at the end of the 20-day exposure, and 4) MITC-induced DNA synthesis/epithelial cell proliferation was not sustained 4 weeks post-exposure indicating a return to normal epithelial cell turnover (no sustained increase in DNA synthesis and cell proliferation).	[redacted] (2020) [redacted] DRAR: **B.6.8.1.2.1/04**

Mouse			
Sub-acute **28-day mouse study** OECD TG 412 (2018) Broncho-alveolar lavage and ophthalmological examination not performed GLP compliant Mice, Crl:CD-1 (male and female); B6C3F1 (female) 5/sex/group	MITC Batch n°: 56198PJV Purity 99.7% Inhalation (whole body) Dose levels: 0, 5, 20, 40 and 80 ppm (corresponding to 0, 15, 60, 119 and 235 mg/m³) (corresponding to 0, 4.21, 16.15, 32.05, 63.21 mg/kg b.w./d 4-week period for 6 hours per day (5 days/week; up to a total of 20 exposures) Vehicle = filtered air	Exposures were terminated after the first exposure for the 80 ppm group of B6C3F1 mice and following the third exposure for the 80 ppm CD-1 mice due to moribundity and clinical signs of toxicity. As a result, all surviving animals at 80 ppm were euthanised on study day 2. All surviving CD-1 and B6C3F1 mice were euthanised on the day following the twentieth exposure (study day 26). There were 2 test substance-related early deaths (each one of the 80 and 40 ppm group) **CD-1** 80 ppm (= 235 mg/m³), 40 ppm (= 119 mg/m³) and 20 ppm (= 60 mg/m³) clinical observations e.g. hypoactivity, hyperactivity, intermittent tremors, complete and/or partial closure of eyes, impaired muscle coordination 40 ppm (= 119 mg/m³) ↓ BW and food consumption, small spleen and thymus; lower liver, spleen, brain (females) and thymus weights microscopic findings (high incidence, severity: mild to moderate) in the nares, nasal levels I, II, III, and IV, in the larynx, trachea, and lungs 20 ppm (= 60 mg/m³) ↓ BW and food consumption lower liver, spleen and thymus weights microscopic findings (high incidence, severity: mild to moderate) in the nares, nasal levels I, II, III, and IV, in the larynx, trachea, and lungs 5 ppm (= 15 mg/m3) partial closure of the eyes and hyperactivity microscopic findings (low incidence, severity: minimal) in the nares, nasal levels I, II, III, and IV, in the larynx, trachea, and lungs **B6C3F1** 80 ppm (= 235 mg/m³), 40 ppm (= 119 mg/m³) and 20 ppm (= 60 mg/m³) clinical observations e.g. hypoactivity, hyperactivity,intermittent tremors, complete and/or partial closure of eyes, impaired muscle coordination 40 ppm (= 119 mg/m³) ↓ BW and food and water consumption Small thymus; lower spleen and thymus weights 20 ppm (= 60 mg/m³) ↓ BW and food consumption lower spleen and thymus weights 5 ppm (= 15 mg/m3) partial closure of the eyes and hyperactivity lower spleen and thymus weights **NOAEL** = <5 ppm (= <15 mg/m3) for both strains **Critical effects at LOAEL**: **Local:** ↑histopathological alterations in the respiratory tract (nasal squamous epithelium hyperplasia) **Systemic**: CD-1: ↑clinical signs, ↓body weight gain (>20%), ↓thymus weight.	[redacted] (2013) [redacted] DRAR: **B.6.8.1.2.1/03**

Sub-chronic 90-day mouse study OECD 413 (2018) GLP compliant Mice, Crl:CD1(ICR) 10/sex/group	MITC Batch n°: 56198PJV Purity 99.7% Inhalation (whole body) Dose levels: 0, 1, 5 and 20 ppm (corresponding to 0, 3, 15 and 60 mg/m³) 13-week period for 6 hours per day (5 days/week; up to a total of 65 exposures) Vehicle = filtered air	No MITC-related deaths, haematology alterations, or macroscopic findings were observed. <u>20 ppm (= 60 mg/m³)</u> ↓ BW and food consumption Clinical observations:hypoactivity, hyperactivity, intermittent tremors, complete and/or partial closure of eyes, standing posture during exposure and laboured respiration impaired muscle coordination. microscopic findings in nasal sections, larynx and trachea, lungs and livers <u>5 ppm (= 15 mg/m³)</u> Clinical observations: hypoactivity, hyperactivity, intermittent tremors, complete and/or partial closure of eyes, standing posture during exposure and laboured respiration, impaired muscle coordination microscopic findings in nasal sections, larynx and trachea (males) <u>1 ppm (= 3 mg/m³)</u> No test substance-related findings **NOAEL** = 1 ppm (= 3 mg/m³) for upper (nasal cavity and larynx) and lower (trachea) respiratory tract **NOAEL** = 5 ppm (= 15 mg/m³) for systemic effects **Critical effects at LOAEL**: **Local**: microscopic findings in the respiratory tract **Systemic**: ↓body weight (gain), ↓food consumption, ↑clinical signs, haematological (↓WBC and ↑neutrophil counts) and clinical-chemical (↑bilirubin), ↓spleen and ↓thymus weights, and ↑microscopic findings in the nasal cavity	[redacted] (2013) [redacted] DRAR: **B.6.8.1.2.2/03**

Chronic **2-year mouse study** (12-month time point) OECD 453 (2018) only 6 animals/ sex for the chronic phase; no blood and urine collection was conducted after 3 months of exposure; Haematological examinations did not include mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), prothrombin time, and activated partial thromboplastin time; clinical biochemistry parameters did not include albumin and calcium examination; only one test for hepatobiliary evaluation; urinalysis did not include volume and osmolality or specific gravity; cervix, coagulating gland, lacrimal gland, rectum, and harderian glands not collected and examined; epididymides and uterus not weighed Non GLP compliant Mice, ICR:JCL 70/sex/group (6/sex/group for chronic and /sex/group for carcinogenicity phase)	MITC Batch n°: MS25206 Purity 93.14% Oral (drinking water) Dose levels: 0, 5, 20, 80 and 200 ppm (corresponded to 0.82, 3.3, 11.83 and 25.71 mg/kg bw/day for males and 0.91, 3.66, 13.03 and 29.03 mg/kg bw/day for females) 26- and 52-week chronic phase and 106-week period Vehicle = untreated drinking water	No MITC-related deaths <u>200 ppm (= 25.71 and 29.03 mg/kg bw/day)</u> ↓ BW and water consumption ↓ water intake Clinical signs: raised hair and a dull coat ↓ erythrocyte levels ↓ lymphocyte count ↓ haematocrit levels ↑ segmented leucocyte (male) ↑ reticulocyte count (male) ↑ potassium levels <u>80 ppm (= 11.83 and 13.03 mg/kg bw/day)</u> ↓ BW (male) and water consumption Clinical signs: raised hair and a dull coat ↓ erythrocyte levels ↓ lymphocyte count ↓ haematocrit levels ↑ segmented leucocyte (male) ↑ potassium levels <u>20 ppm (= 3.30 and 3.66 mg/kg bw/day)</u> No test substance-related findings <u>5 ppm (= 0.82 and 0.91 mg/kg bw/day)</u> No test substance-related findings **Systemic NOAEL** = 20 ppm corresponding to 3.3 and 3.66 mg/kg bw/day in males and females, respectively **Carcinogenic NOAEL**: 200 ppm **Critical effects at LOAEL**: **Systemic**: Reduced BW and food consumption at 80 ppm corresponding to 11.83 and 13.03 mg/kg bw/day in males and females, respectively.	[redacted] (1980) [redacted] DRAR: **B.6.8.1.4.2/01**

Chronic **18-month study** OECD 453 (2018) No justification is provided regarding exposure for 5 days per week; water consumption not measured GLP compliant Mice, Crl:CD1(ICR) 50/sex/group	MITC Batch n°: 56198PJV Purity: 97.2-99.7 Inhalation (whole-body) Dose levels: 0, 1, 5 and 15 ppm (corresponding to 0, 3, 15 and 45 mg/m³) 78-week period for 6 hours per day with 5 days/week Vehicle = filtered air	No MITC-related deaths <u>15 ppm (= 45 mg/m³)</u> ↓ BW (~20%) and overall BWG (~70%) ocular findings, including opacity correlated with macroscopic finding of eye opacity; lower spleen (males and females) and thymus (males) weights; nasal lesions, olfactory epithelial degeneration and olfactory epithelial changes in the nasal cavity, cytomegaly and karyomegaly (minimal to mild) and of submucosal inflammation and chronic active inflammation, inflammatory exudate in all nasal levels, proliferative and/or inflammatory lesions were observed in the larynx, trachea, and eye <u>5 ppm (= 15 mg/m³)</u> ↓ BW (~5%) and overall BWG (~15%) Lower spleen weights (males); nasal findings (low incidence, severity: minimal to mild) <u>1 ppm (= 3 mg/m³)</u> No test substance-related findings **Local NOAEL:** **Systemic NOAEL** = 1 ppm (= 3 mg/m³) outside the respiratory tract and for local effects **Carcinogenic NOAEL** = 15 ppm (= 45 mg/m³) **Critical effects at LOAEL**: **Local:** ↑respiratory and transitional hyperplasia of nasal epithelium; **Systemic:** ↓body weight, ↓body weight gain, ↓spleen weights (♂)	[redacted] (2015) [redacted] DRAR: **B.6.8.1.4.2/02**
Dog			

Sub-chronic 90-day dog study OECD 409 (1998) Stability (expiry date), air changes per hour and length of the photoperiod not being determined; the temperature range was reported between 6 and 28 °C GLP compliant Dog, Beagle 4/sex/group	MITC Batch n°: BX 340178 AD 11308 / BX 340178 AD 11328 Purity 95.96% Oral (gavage) Dose levels: 0, 0.04, 0.4 and 2.0 mg/kg bw/day Twice daily for 13-week period Vehicle = corn oil	No MITC-related deaths. <u>2.0 mg/kg bw/day</u> Vomiting, excessive salivation occasionally observed during the final 7 weeks of dosing ↑ mean activated partial thromboplastin (males) ↓ total protein and/or total globulin ↓ all four globulin fractions ↓ calcium levels white catarrhal material adherent to the stomach mucosa and generalised pallor of skeletal muscle ↓ testes weights ↑ pancreas weights (females) Hepatocyte vacuolation and lipid deposition and thymic involution <u>0.4 mg/kg bw/day</u> Vomiting ↓ testes weights ↑ pancreas weights (females) Hepatocyte vacuolation and lipid deposition and thymic involution <u>0.04 mg/kg bw/day</u> Vomiting, No MITC-related findings **NOAEL** = 0.4 mg/kg bw/day **Critical effects at LOAEL**: **Systemic** (2.0 mg/kg bw/d): increased incidences/frequency of clinical signs (vomiting, excessive salivation), ↓bodyweight, haematological findings, single blood chemistry parameters, liver coarse texture, ↑thymic involution and ↑severity of lipid periportal depots and vacuolation.	[redacted] (1986) [redacted] DRAR: **B.6.8.1.2.2/01**

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
90-day, oral (capsule feeding) dog study	Metam sodium Batch n°: BAS/005/00 N Purity 43.15%	Oral, gelatine capsules Dose levels: 0, 1, 5, 10 mg/kg bw/d 90-days, daily (7 days/week).	**1 mg kg bw/day:** no relevant findings **≥5 mg kg bw/day:** significant elevation of transaminase and alkaline phosphatase, and γGTactivity, total bilirubin level, clinical signs (vomiting), liver necropsy findings and histopathological evidence of hepatitis and biliary inflammation. **10 mg kg bw/day:** haematological findings (RBC and WBC modofications), ↓bw gain, clinical signs (salivation, jaundice), mortality.	[redacted], **1992**

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Metam (incl. -sodium and -potassium):

[redacted] (1991), [redacted], DRAR: B.6.3.2.1/01
Metam sodium was administered orally for a period of 90 days via drinking water to 4 groups of male and female C57BL/10JfAP/Alpk rats at concentrations of 0.018, 0.088, 0.35 and 0.62 mg/mL (corresponding to 0.79, 4.48, 36.05, 60.36 mg/kg bw/day for males and 1.05, 5.82, 38.12, 64.27 mg/kg bw/day for females). The adapted concentrations were taken from the Californian EPA assessment (2004), were the decay of metam sodium was taken into account.

A **NOAEL** was established at 0.018 mg/mL **= 0.79 mg/kg bw/d,** based upon haematological findings (↓haemoglobin, haematocrit and RBC count) and histopathological changes, apparent in the urinary bladder (eosinophilic granules in transitional epithelial cells of the urinary bladder), observed at the LOAEL of 0.088 mg/mL (= 4.5 mg/kg b.w./d) and above. At 0.35 mg/mL and above, impaired body weight and body weight gain, ↓food and water consumption, ↑relative liver weight, pale liver showing accentuated lobular pattern and mucosal hyperplasia of urinary bladder was observed. The nasal cavity was not affected in the mouse, contrarily to what has been observed in the rat.

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (mice) = 0.8 mg/kg bw/d (metam sodium))

[redacted]. (1991), [redacted], B.6.3.2.2/01
Metam sodium was administered orally for a period of 90 days via drinking water to 3 groups of male and female Alpk:APfSD rats at concentrations of 0.018, 0.089, and 0.443 mg/mL, corresponding to 0.49, 3.1, 18.51 mg/kg bw/day for males and 0.73, 3.68, 21.05 mg/kg bw/day for females, respectively.

A **NOAEL** can be set at **0.018 mg/mL (= 0.49mg/kg bw/d**) taking into account the ↓body weight and ↓body weight gain resulting from ↓water consumption (supported by ↓urinary volume and ↑specific gravity), altering food consumption at 0.089 mg/mL (= 3.1 mg/kg bw/d) and above.
At the top-dose (0.443 mg/mL = 18.5 mg/kg b.w./d) adverse effects included ↓body weight (>20%), altered clinical chemistry and urinalysis, and overt histopathologic alterations in the olfactory epithelium.
The established NOAEL disregards sporadic effects of ↑water consumption, RBC count, and slight ↑triglyceride level at the lowest dose, but overall, taking into account the magnitude of the cited observed adverse effects, and the fact that subtle modifications at the mid-dose confirm the substance-related adverse findings at the top-dose, the proposal of the NOAEL at the low dose remains justified.

This endpoint was previously agreed (EFSA Journal 2011;9(9):2334): NOAEL (rat) = 0.5 mg/kg bw/d (metam sodium))

[redacted]. (1983), [redacted]; Re-Examination of liver sections: [redacted]. (1992) [redacted], DRAR B.6.3.4.2/01
The objective of this study was to evaluate potential toxic effects of VAPAM Technical when administered via whole-body inhalation to Sprague Dawley rats for 6 hours per day on a 5-day per week basis for 65 days at 0, 6, 32 and 160 mg/m3 (18 animals of each sex were selected per dose group).

Local NOAEL was considered <6.5 mg/m³ or **<1.75 mg/kg bw/d**, based upon histopathological nasal passage findings: ↑lymphocytic rhinitis and ↑hyperplasia of mucogenic epithelium (top-dose findings included ↑lung histiocytosis and ↑mucigenic cysts).
The **systemic NOAEL** was set at 6.5 mg/m³ or = **1.75 mg/kg bw/d**, based upon ↓b.w. gain, ↓food consumption, ↑ALP and ↑liver mononuclear cell infiltration. Top-dose local/systemic effects (160 mg/m³) included effects in the liver: ↑rel. weight and ↓albumin (liver damage), in the stomach: ↑glandular duct ectasia, erosive/ ulcerative gastritis, urinary tract transitional epithelium hyperplasia/ metaplasia and in the kidney: ↑relative weight, ↑pyelitis, as well as in the adrenals and lungs, showing ↑relative weight.

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (rat) = 6.5 mg/m³ corresponding to 1.75 mg/kg bw/d (metam sodium).

[redacted]. (1994), [redacted], DRAR B.6.7.1/03
The objective of the study was to perform an overall neurotoxicological evaluation of the Alpk:APfSD rats after 13 weeks of administration of metam sodium. Groups of 12 male and 12 female rats were administered drinking water containing metam sodium at 0.02, 0.06 and 0.2 mg/mL for 13 weeks.

Under these experimental conditions, metam sodium at dose levels up to 0.2 mg/mL, equivalent to 14.7 mg/kg b.w./d, did not show signs of subchronic neurotoxicity.
A slight significant decrease in both body weight and food consumption was observed at the lowest dose onwards. Therefore, the **systemic toxicity NOAEL** was **< 2.0 mg/kg b.w./d**. In the regular OECD-compliant 90d study in rats of the same strain a NOAEL was identified at 0.5 mg/kg b.w./d.
The **subchronic neurotoxicity NOAEL = 14.7 mg/kg bw/d**.

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): repeated neurotoxicity NOAEL 14.7 mg/kg bw/d (metam sodium).

[redacted]. (1992), [redacted], B.6.3.2.3/01; [redacted]. (1993), [redacted], B.6.3.2.3/02
Metam sodium was administered orally for a period of 90 days via gelatin capsules to groups of 4 male and 4 female Beagle dogs at concentrations of 1, 5, and 10 mg/kg bw/day.

A NOAEL = **1 mg/kg b.w./d** was proposed, taking into account clinical signs of toxicity (salivation, food regurgitation), altered haematological and clinical chemistry parameters, increased urinary bladder cell mitosis, hepatitis and biliary inflammatory cell infiltration, at the LOAEL = 5 mg/kg b.w./d and above.
Top-dose animals (10 mg/kg b.w./d) suffered in addition jaundice (supported by urinalysis abnormalities), increased kidney weight and increased mortality.
Details of the severe hepatic lesions observed in the 90d dog study are available in the core assessment of the a.s. in Vol.3 B.6.3.2.3 (tables B.6.3.2.3/01-1 through -10), and summarised here below:

Table 48b Adverse findings 90-day dog study with metam-sodium at termination indicating serious liver toxicity ([redacted],1992)

Sex	♂				♀			
Dose [mg/kg bw/day]	**0**	**1**	**5**	**10**	**0**	**1**	**5**	**10**
Clinical chemistry								
	Alanine transaminase activity (ALT)							
mean [IU/L]	28.5	29.0	**429.3****	**711.3****	29.8	**134.5***	**207.5***	**266.7****
± SD	3.8	8.5	451.6	283.2	9.0	213.2	201.7	28.4
Δ%		1.8	**1406.3**	**2395.8**		**351.3**	**596.3**	**795.0**
	Aspartate transaminase activity (AST)							
mean [IU/L]	20.8	19.5	**50.3***	**115.3****	21.0	**28.3**	**30.3**	**43.7***
± SD	7.4	4.0	23.5	72.2	2.9	24.2	14.4	15.2
Δ%		-6.3	**141.8**	**454.3**		**34.8**	**44.3**	**108.1**
	Alkaline phosphatase activity (ALP)							
mean [IU/L]	155	160	**524***	**1177****	141	157	**377**	**588****
± SD	26	10	423	**374**	18	24	340	178
Δ%		3.2	**238.1**	**659.4**		11.3	**167.4**	**317.0**

Sex	♂				♀			
Dose [mg/kg bw/day]	**0**	**1**	**5**	**10**	**0**	**1**	**5**	**10**
	Gamma-glutamyl transferase activity (γGT)							
mean [IU/L]	0.8	0.5	1.5	**4.7****	0.8	0.8	1.3	**4.0***
± SD	1.0	1.0	1.7	0.6	1.0	1.0	2.5	5.2
Δ%		-37.5	**87.5**	**487.5**		0.0	**62.5**	**400.0**
	Total bilirubin (TB)							
mean [mg/100 mL]	0.20	0.20	0.27	**5.20****	0.20	0.20	**0.27**	**0.73**
± SD	0.00	0.00	0.10	4.29	0.00	0.00	0.15	0.92
Δ%		0.0	**35.0**	**2500.0**		0.0	**35.0**	**265.0**
Gross pathology liver								
accentuated lobular pattern	0	0	0	**3**	0	0	**1**	**3**
pale	0	0	0	**3**	0	0	0	**2**
depressed red areas	0	0	**1**	**2**	0	0	**1**	**2**
Histopathology liver								
Total hepatitis	0	0	**3**	**4**	0	0	**2**	**4**
biliary inflammatory cell infiltration	0	0	**1** (slight)	**1** (marked)	0	1 (minimal)	1 (minimal)	**1** (marked)

Statistically significant modification: Student´s t-test;* $p \leq 0.05$,** $p \leq 0.01$; Values presented as mean±s.d. or as number affected animals; Δ% = related to the respective control (%); N=4/sex/dose, except N=3 in top-dose animals at week 12 (♀) and week 13 (♂,♀).

In summary, metam sodium, orally administered via capsules to ♂ and ♀ Beagle dogs for 90 days, caused treatment-related hepatitis in both sexes dosed with 5 mg/kg bw/day and above, which resulted in the early termination of 2 dogs in the top dose group due to adverse clinical signs. The onset of this treatment-related hepatitis showed a clear dose and time relationship. Other modified parameters were marked increases in ALT, AST and ALP. The progressive inappetence and weight loss occurred later and coincided with increases in plasma γGT activity, total bilirubin and jaundice. Gross pathology revealed accentuated liver lobular pattern with pale appearance or depressed red areas. Marked hepatitis was seen microscopically. There was a variety of changes, which were considered secondary to the hepatotoxicity or indicative of generalised toxicity or poor clinical conditions. Taking into account the clinical signs, and both liver and kidney/urinary bladder adversity observed at the **LOAEL of 5 mg/kg bw/day** a NOAEL of 1 mg/kg bw/day was set.
This LOAEL is below the guidance value of **10 mg/kg bw/day** , justifying the proposal of classification of metam-sodium as specific target organ toxicant following repeated exposure (STOT-RE 1).

A reversal of both macroscopic and microscopic changes in the liver had taken place between cessation of treatment with metam sodium and termination of a supplemental study at 10 mg/kg b.w./d, however conducted one only 1 animal/sex exposed to one dose ([redacted], 1993), and thus of very limited impact on the final conclusion.

[redacted] (1994), [redacted], B.6.3.3/01
Metam sodium was administered orally for a period of 1 year via gelatin capsules to groups of 4 male and 4 female Beagle dogs at concentrations of 0.05, 0.1, and 1 mg/kg bw/day.

In this study, hepatotoxicity was more evident in female dogs as suggested by increased ALT and some liver histopathological findings. The **NOAEL = 0.1 mg/kg bw/d.** Overall, the one-year dog study was considered to provide the most relevant metam medium-term NOAEL.

EU-agreed endpoint (EFSA Journal 2011; 9(9):2334): NOAEL (dog) = 0.1 mg/kg bw (metam sodium)

[redacted] (1979), [redacted], DRAR: B.6.3.4.2/01
Metam Fluid (metam-sodium), which was applied to the intact and abraded dorsal skin of rabbits at dose levels of 31.25, 62.50 and 125.0 mg/kg bw/day, was administered to groups of 5 ♂ and 5 ♀ rabbits (White Russians) for an exposure time of 8 hours over 21 days.

No systemic toxicity was observed. Local skin reactions such as erythema edema and rhagades and epidermis-dermatitis were seen at 62.5 and 125 mg/kg bw/d. **NOAEL for local effect**s = **31.25 mg/kg bw/d**.

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (rabbit) = 31.2 mg/kg bw/d (metam sodium).

The study from [redacted] (1979) on subacute inhalation toxicity ([redacted]) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness the study is nevertheless summarised in the MCA 5 dossier (KCA 5.3.3/03). The results of this study are in line with the results of the studies relied upon. Therefore, the study from [redacted] was not considered for the hazard and risk assessment and are not further used in the CLH dossier for classification purposes.

MITC:

The repeated-dose toxicity of MITC by inhalation has been investigated in 28-day, 90-day and chronic studies in rats and mice. Additionally, chronic studies were conducted on rats and mice following oral administration of MITC (drinking water) as well as a 90-d study on dogs treated orally via gavage. Several animals died after inhalation of the high doses *i.e.* 240 mg/m³ (28-day study, rats and mice), 120 mg/m³ (28-day study, mice) and 60 mg/m³ (12-month interim kill, rats). Beside the respiratory tract and local effects (ocular findings, including opacity), no specific target organ was identified neither after oral application nor inhalation. After oral application of MITC to rats and mice, mild system toxic effects were noted after 12-month of treatment.

Rat studies

[redacted] (1987), [redacted], DRAR B.6.8.1.2.1/01
In a subacute inhalation toxicity study MITC was administered as vapour to groups of 5 male and 5 female Wistar rats for 28 days with target concentrations of 5, 20 and 100 mg/m³.

At the top-dose of 100 mg/m³, lung weight was increased, and was associated with bronchopneumonia and epithelial proliferation in bronchi and bronchiole. Elevated bilirubin and thromboplastin time were also noted.
The **local NOAEL** was considered **<5 mg/m³ = <1.35 mg/kg bw/d**, due to a slight increase of focal atrophy at 5 mg/m³ and general atrophy at 20/100 mg/m³, indicating an increase of severity of the lesion.
The **systemic NOAEL** was considered **5 mg/m³ = 1.35 mg/kg bw/d,** based on ↓ body weight (gain), ↑ clinical signs, ↑ non-focal atrophy olfactory epithelium, ↑ neutrophils).

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (rat) = 5 mg/m³, corresponding to 1.35 mg/kg bw/d (MITC).

[redacted] (2011), [redacted] DRAR B.6.8.1.2.1/02
In a 4-week inhalation range-finding study, Crl:CD(SD) rats were exposed (6-hour inhalation exposure period, whole-body) to MITC at concentrations of 0, 5, 20, 40, and 80 ppm (corresponding to 0, 15, 60, 120 and 240 mg/m³).

There was mortality and early termination of the 80 ppm group males and females on study day 16. Prior to death, these animals revealed severe signs of toxicity, accompanied by body weight loss and low food consumption. However, cumulative body weight change was meaningfully and dose-dependently reduced at the lowest dose onwards. It is noted that reduced thymus weight was also observed at the lowest dose, as well as the isolated histopathological findings. Most substance-related microscopic findings were observed in the nasal cavity (levels I trough VI), larynx, trachea and lungs of all animals (♂ and ♀) exposed at 20 and 40 ppm, although it is noted that a single incidence of squamous metaplasia and epithelial regeneration of the larynx was observed at the lowest dose of 5 ppm.

Based on effects on survival at 80 ppm, a dose-related statistically significant reduction in body weight and/or body weight gains at 20, 40 and 80 ppm, respectively, and considering increased incidences and the severity of portal of entry findings in the respiratory tract at 20 and 40 ppm, exposure levels of 1, 5 and 15 ppm, respectively were selected for the subsequent main 90-day inhalation toxicity study with MITC in rats.
Therefore, a **NOAEL for local toxicity** can be set to **5 ppm (= 15 mg/m³ or 4 mg/kg bw/d**), based upon the histopathological alterations in the respiratory tract at the next-higher doses of 20 ppm and above (= 60 mg/m³ or 16 mg/kg bw/d). The **systemic NOAEL** is proposed at **<5 ppm (= <15 mg/m³ = <4 mg/kg bw/d)**, based upon clinical signs, decreased body weight gain and thymus weight (>10%).

[redacted] (2012), [redacted], DRAR B.6.8.1.2.2/02
♂ and ♀ Crl:CD(SD) rats were exposed to MITC vapours actual concentrations of 0, 1.02, 5.05 and 15.06 ppm (equivalent to 0, 3.06, 15.15 and 45.18 mg/m³) once daily on a 5 d/week - 6 h/d basis (minimum of 65 total exposures).

No treatment-related changes in body weight and food consumption were noted. Alterations in most organ weights were not related to exposure concentration or not correlated to histopathology (except for the liver findings).
Alterations in haematology and coagulations parameters were observable in ♀ at the top-dose.
Adverse findings in the different regions of the respiratory tract were observed at the top-dose of 15 ppm in both ♂ and ♀. In nasal epihelium at level II, an increased incidence of minimal (5 ppm and above) to mild (top-dose) acute inflammation was observed in ♀, along with level III and IV minimal olfactory epithelium single cell necrosis.
Nasal epithelial findings in a single ♂ in the 1 ppm group, without concomitant dose-related effects at the next-higher dose could be considered to be non-adverse. No significant histopathological effects on the lungs or trachea were observed.

In this rat 90d- rat inhalation study with MITC, a local **NOAEL = 1.02 ppm (**equivalent to **3.06 mg/m³** or **0.4 mg/kg b.w./d)** can be deduced, on the basis of adverse histopathological effects at the next-higher dose.
The systemic **NOAEL** = **5.05 ppm** (equivalent to **15 mg/m³** or **4 mg/kg b.w./d)** is based on effects on haematology and coagulation parameters (↑APTT, ↑neutrophils), increased liver and decreased thymus weight at 15 ppm **(**equivalent to **45 mg/m³** or **12 mg/kg b.w./d)**.

[redacted] (1984), [redacted], DRAR B.6.8.1.4.1/01
Methyl isothiocyanate (MITC) was administered orally for a period of 104 weeks via drinking water to 3 groups of CD rats at 2, 10, and 50 ppm. Exposure concentrations of 2, 10 and 50 ppm corresponded to 0.08, 0.37 and 1.60 mg/kg bw/day for males and 0.12, 0.56 and 2.65 mg/kg bw/day females respectively.

Reduced body weight of ♂ rats, was more important at top dose at the end of the study period, white blood cell parameters, histopathological findings such as bone marrow hyperplasia, increased kidney microcalculi, liver effects, and spleen hyperplasia/ increased haematopoiesis were reported at 50 ppm (1.6 mg/kg bw/d) and could be related to MITC exposure.
A **systemic NOAEL= 10 ppm (0.44 mg/kg bw/d)** is proposed.
MITC is not carcinogenic under these experimental conditions, and a carcinogenicity NOAEL = **50 ppm (1.60 mg/kg bw/d)** is set accordingly.

[redacted] (2015), [redacted] DRAR B.6.8.1.4.1/02
Methyl isothiocyanate (MITC) was administered to Crl:CD(SD) rats for a period of 104 weeks via whole-body inhalation exposure for 6 hours per day at target exposure concentrations of 0.5, 5, and 20 ppm.

No statistically significant effect on the survival was noted for neither ♂ nor ♀ rats in any group at termination. However, the top-dose animals beared squamous cell carcinoma and anaplastic carcinoma of the nose, as well as lung necrotising or suppurative inflammatory lesions, secondary to MITC-related injury. While survival rate was equally distributed among the treated animals, the cause of death in these top-dose group was mainly the presence of nasal tumours.
Thin appearance, associated with lower body weight and food consumption, also had a higher occurrence and incidence in the 20 ppm ♂ and ♀. In the 5 ppm group and above, rales and laboured respiration were observed with a slightly higher occurrence and/or incidence in ♂ and ♀. MITC exposure-related palpable masses were located on or near the nose in top-dose ♂ and ♀.
Based on mean body weights and cumulative body weight gains, the high-concentration of 20 ppm corresponded to or exceeded the maximum tolerated dose (MTD).
Treatment-related clinical and anatomic pathology findings noted prior to or at the time of the chronic toxicity evaluation (study week 52) included changes in haematology and clinical chemistry parameters, organ weight effects (higher lung weights, secondary higher adrenal gland weights, and secondary stress-related lower spleen and thymus weights) and microscopic lesions in the nasal cavity, larynx, trachea, and lung. These lesions resulted from direct contact injury to mucosal epithelium by MITC, had a higher incidence and were most severe at the top-dose.

The systemic **NOAEL** (*i.e.* effects mostly outside the respiratory tract) is **5 ppm,** on the basis of decreased body weight (gain), haematology and clinical chemistry parameter modifications, and organ weight effects.

Non-neoplastic histopathological findings along the upper airway tract at 0.5 ppm and above were squamous and respiratory epithelial metaplasia, and olfactory epithelial degeneration in nasal tissues, and epithelial hyperplasia and squamous metaplasia in the larynx. Other non-neoplastic nasal, laryngeal, tracheal and lung lesions were considered adverse in the 5 and/or 20 ppm group.
Other treatment-related microscopic findings outside of the respiratory tract were in the olfactory bulb (brain) and eyes. Olfactory bulb effects were a direct consequence of nasal olfactory epithelial degeneration and atrophy, while adverse effects in the eyes were a consequence of direct contact with the irritant MITC.

The **local NOAEL** is **< 0.5ppm,** based upon increased incidences of squamous metaplasia and olfactory epithelial degeneration in the nasal epithelium, and epithelial hyperplasia and squamous metaplasia on the larynx. It seems evident that the observed nasal effects at the lowest dose are part of a gradient which incidence and severity grade increased at the next-higher doses. Toxicologically significant local effects (portal of entry) were observed clinically in the eyes (opacity and bilateral keratitis in 20 ppm group) and microscopically in the nasal tissues, larynx, trachea, lungs, olfactory bulbs, and eyes.

MITC exposure-related *neoplastic findings* were found in the 20 ppm group ♂ and ♀ and included malignant and benign nasal tumours and a single benign papilloma in the lung (1♂).

The **carcinogenicity NOAEL is 5 ppm,** based upon increased incidences of squamous cell carcinoma (25%) and anaplastic carcinoma (about 3%) in both ♂ and ♀, adenoma (5%) and papilloma (10%) in the ♂ **at the top-dose of 20 ppm**. Invasive extensions of the carcinoma were found at the level of neighbouring tissues, as well as metastases in the mandibular lymph node. In addition, a single lung papilloma (♂) is observed at this dose as well.

[redacted] (2020), [redacted], DRAR b.6.8.1.2.1/04
The objective of this study was to evaluate whether methyl isothiocyanate (MITC) exceeded the maximum tolerated dose (MTD) when administered at 20 ppm via whole-body inhalation to male Sprague Dawley rats (Crl:CD(SD)) for 6 hours per day. Further, this study was performed to elucidate the possible mode(s) of action (MoA) involved in development of nasal tumours in Sprague Dawley rats following MITC exposure via the inhalation route.
Groups of 8 male rats were exposed to 0, 0.5, 5 and 20 ppm MITC for 1 day (Subset A), 5 days (Subset B), 4 weeks (Subset C) and 4 weeks followed by a 4-week recovery period without exposure (Subset D). Each animal of the respective dose group and Subset was exposed on 5 days per week for 6 hours per day. A group exposed to filtered air was included to serve as control. All animals were observed for mortality at least twice daily and for clinical signs twice a day on exposure days and daily on non-exposure days. In addition, detailed clinical examination, individual body weights and food consumption were recorded weekly during the study period.
2-2.5 hours prior to sacrifice, all animals were injected the cell proliferation marker BrdU at a concentration of 50 mg/kg bw. Haematology and clinical chemistry parameters were analysed on the days of necropsy. Gross necropsy and a histopathological examination of the nasal tissue were conducted.

Overall, the adverse histopathological findings (increased incidence of inflammation and hyperplasia) at 5 ppm onwards should be regarded toxicologically meaningful, also taking into account the effects on b.w. and the elevated DNA-replication marker in the nasal epithelial cells at 5 ppm and above.
Therefore, a tentative **NOAEL = 0.5 ppm**, corresponding to 1.5 mg/m³ is established, based on the b.w. effects and histopathological findings, supported by evidence of increased DNA replication at 5 ppm and above, corresponding with approximately **1.5 mg/m³** or **0.40 mg/kg b.w./d.**
It may be suggested that plausible key events early in the pathogenesis of MITC-induced nasal tumours, found in a subsequent carcinogenicity in the rat at high exposure concentrations include:
(i) transient nasal epithelial cell death,
(ii) persistent regenerative epithelial cell proliferation and DNA synthesis (increased cellular turnover) with sustained inhalation exposures to MITC.

The irritant effect of MITC leading to an increased epithelial cell proliferation, was a common finding in 20 ppm, an occasional finding in 5 ppm- but barely perceptible in 0.5 ppm-exposed rats, at the end of the 20-day exposure. These proliferative non-neoplastic lesions in targeted intranasal sites of toxicity may be anticipatory signs of preneoplastic lesions of nasal cancers, especially squamous cell carcinomas, that develop in rats chronically exposed to high concentrations of MITC.

Mouse studies

[redacted] (2013), [redacted], DRAR B.6.8.1.2.1/03
In a range-finding test in CD -1 Mice and Female B6C3F1 Mice, the animals were exposed (whole-body) to MITC at concentrations of 0, 5, 20, 40, and 80 ppm, via a 6-hour inhalation exposure period for 4 consecutive weeks.
There was mortality and early termination of both sexes of the 80 ppm group of CD-1 and $B_6C_3F_1$ animals on study day 2. Prior to death, these animals revealed severe signs of toxicity during the clinical observation procedures.
Test substance-related clinical observations were noted at exposure levels ≥20 ppm in CD-1 mice and at exposure levels >20 ppm in $B_6C_3F_1$ mice. Test substance-related clinical observations noted in the 5 ppm groups of mice were limited to partial closure of the eyes (both strains) and hyperactivity (CD-1).
In both mouse strains, a dose-related statistically significant reduction in body weight and/or body weight gains was seen at exposure levels of 20 ppm and 40 ppm, but in the CD-1 strain a lower cumulative body weight gain was already evident at the lowest dose onwards. In the 40 ppm group of $B_6C_3F_1$ mice, test substance-related lower water consumption was noted.
Various absolute and relative organ weight changes were also seen at 20 ppm and above. Of note, organ weight decreases were also observed at 5 ppm onwards for thymus (CD-1, $B_6C_3F_1$) and spleen ($B_6C_3F_1$), in a dose-responsive manner.
In CD-1 mice, severe effects on portal of entry (nose, larynx, trachea, and lungs) were seen mostly at 20 ppm and above, although some effects were also observed at the lowest dose.

Overall, in this study, the local **LOAEL** is set at **5 ppm (= 15 mg/m³),** based upon hyperplasia of the nasal squamous epithelium in the majority of the ♂/♀ animals, and an isolated case of minimal respiratory epithelium and larynx regeneration in 1♀ animal from the lowest dose onwards. Along with abovementioned b.w. change and organ weight decreases, RMS therefore considers the lowest dose of 5 ppm a systemic LOAEL rather than a NOAEL.
The exposure levels selected for the subsequent 90-day inhalation study (1, 5 and 20 ppm) are supported. It is noted that exposure levels of 1, 3, and 10 ppm were selected for a specific immunotoxicity inhalation study with MITC in $B_6C_3F_1$/Crl mice, although it would appear that the CD-1 strain is more sensitive overall.

[redacted] (2013), [redacted], DRAR B.6.8.1.2.2/03
Crl:CD1(ICR) mice were exposed to MITC via a 6-hour inhalation exposure period for 13 consecutive weeks at 1, 5, and 20 ppm (corresponding to 3, 15, and 60 mg/m³).

Clinical signs of toxicity at 5 ppm and above were characterised by complete and/or partial closure of the eyes, standing posture, and laboured respiration, and at the top-dose hypo- or hyperactivity.
Statistically significantly lower food consumption and body weights/body weight gains were noted in ♂ and ♀ of the 20 ppm group throughout the study.
On terminal investigation, an increase of both absolute and differential count of neutrophils was observed at 5 ppm (♂) and above (♂,♀), which was related to the slight inflammatory changes at these dose-levels. WBC counts, and more specifically, lymphocyte counts were also low in the top-dose animals (♂,♀). Platelet counts were increased at the top dose. It is noted that other coagulation parameters, such as partial thromboplastin time (APTT) and prothrombin time (PT) were not evaluated in the present study, while they occasionally show increases in other studies.
Total bilirubin and cholesterol values were significantly increased in the 20 ppm top-dose group in both sexes of mice. These increases were not correlated to histopathological findings in the liver, but increased bilirubin was observed in a 28-day rat study, and therefore, the association with the treatment cannot be excluded, although the adverse character remains unclear.
Notable weight changes were observed for thymus and spleen, for which modifications have been observed in other studies.
The primary direct test substance-related changes most prominent in the 20 ppm group were local effects in the nasal cavity, considered to be the result of the severely irritating properties of MITC, which were considered adverse. On histopathological examination of ♂ and ♀ mice findings which were made in all nasal cavity sections included squamous epithelial hyperplasia, hyperkeratosis, regeneration of respiratory and olfactory epithelium, cytomegaly and karyomegaly of squamous and/or respiratory and olfactory epithelium, squamous and respiratory metaplasia, hyaline droplets, subacute inflammation, squamous epithelial erosion, and inflammatory exudate.
At 5 ppm and above, changes in respiratory epithelium of the trachea and larynx included minimal single cell necrosis or minimal to mild epithelial hyperplasia.
In the nasal epithelium effects included minimal to mild hyperkeratosis, minimal to severe cytomegaly/ karyomegaly, minimal to moderate/severe subacute inflammation, mild to moderate nerve bundle atrophy and minimal to moderate regeneration of the respiratory epithelium.
The incidence and severity of the histopathological lesions were higher in the anterior nasal sections than in the more caudal sections.
In this 90d mouse- inhalation study with MITC, a **local NOAEL** was set at the dose level of **1 ppm** (equivalent to 3 mg/m³, converted to an internal dose of about 0.88 mg/kg b.w./d) based on histopathological findings noted at dose levels of about 5 ppm, based upon treatment-related microscopic findings in the nasal cavity at 5 ppm and above.
The **systemic toxicity NOAEL** is set at the dose level of **5 ppm** (equivalent to 15 mg/m³, converted to an internal dose of about 4.4 mg/kg b.w./d), based upon decreased body weight (gain), food consumption, clinical signs, haematological (lower WBC and higher neutrophil counts) and clinical-chemical (elevated bilirubin) findings, decreased liver, spleen and thymus weights, and microscopic findings in the nasal cavity, at the top-dose of 15 ppm (equivalent to 45 mg/m³, converted to an internal dose of about 13 mg/kg b.w./d).

[redacted] (1980), [redacted]
Methyl isothiocyanate (MITC) was administered orally for a period of 106 weeks via drinking water to 4 groups of ICR:JCL mice at concentrations of 5, 20, 80 and 200 ppm.

A NOAEL = 20 ppm (3.3 mg/kg bw/d) is proposed, taking into account the different slight effects seen at 80 ppm (12 mg/kg bw/d) such as the increased incidence of clinical signs, slight decreased body weight and body weight gain, slight effects in blood and altered organ weights. Based on this study, MITC is not carcinogenic after administration in the drinking water in mice.
A **carcinogenicity NOAEL = 200 ppm (25 mg/kg bw/d)** may be proposed.

[redacted] (2015), [redacted], DRAR B.6.8.1.4.2/02
Methyl isothiocyanate (MITC) was administered to Crl:CD1(ICR) mice for a period of 78 weeks (5 days per week) via whole-body inhalation exposure for 6 hours per day at target exposure concentrations of 1, 5, and 15 ppm.

Although the 15 ppm high concentration exceeded the MTD (based on low mean body weight and cumulative body weight gain), there were no MITC exposure-related early deaths and no negative effects on survival.
The **systemic NOAEL (i.e. effects outside the respiratory tract) = 1 ppm**, based on lower mean body weight and body weight gain, lower food consumption and decreased spleen weight at 5 ppm (=15 mg/m³) and above.
Toxicologically significant local effects were observed clinically in the eyes (opacity in 15 ppm) and microscopically in the nasal tissues, olfactory bulbs, larynx, trachea and the cornea of the eyes.
In the nasal epithelium, the incidence of both respiratory and transitional epithelial hyperplasia was meaningfully and dose-dependently increased from the lowest dose of 1 ppm onwards, in both ♂and ♀.
Therefore, the **local NOAEL <1 ppm** (<3 mg/m³), on this basis. However, it is also clear that most other histopathological findings were elevated at 5ppm and 15 ppm.

There was a single nasal neoplasm that consisted of a benign, exophytic papilloma in nasal level 1 in a 15 ppm group ♀ at the 18-month terminal necropsy. The papilloma was located on the nasoturbinate in an area of treatment-related respiratory epithelial metaplasia. Although very rare (Brown et al., 1991), spontaneous nasal neoplasms have been reported in mice. It was considered that there was insufficient evidence to indicate that MITC is carcinogenic in the CD-1 mouse. Therefore the **carcinogenicity NOAEL = 15 ppm** (=45 mg/m³).

Dog studies

[redacted] (1986), [redacted]
The 90-day repeated dose oral toxicity of MITC by oral gavage (twice daily) was performed at concentrations of 0 (control group), 0.04, 0.4 and 2.0 mg/kg bw/day in Beagle dogs (4 males and 4 females per group) at 26 to 29 weeks of age at the start of administration.
No mortality occurred. Clinical signs included vomiting and excessive salivation and occurred more frequently at the top-dose of 2.0 mg/kg bw/d.
There was an adverse effect on bodyweight gain for most ♀ receiving 2.0 mg/kg bw/d. There was no effect on food intake, ophthalmoscopy or urinalysis considered to be attributable to the test compound.
Mean activated partial thromboplastin times for ♀ from all treated groups during week 6 and ♂, and ♀ at the top-dose during week 13 were significantly higher than the control value. The mean prothrombin time for ♂ receiving 2.0 mg/kg bw/d was also significantly increased during week 13. There was an effect on serum proteins with significantly decreased total protein and/or total globulin concentrations reported for ♂ and ♀ receiving 2.0 mg/kg bw/day during weeks 6 and 13. Electrophoresis during week 13 revealed decreases in all four globulin fractions (αl, α2, β, γ) for ♂ and/or ♀ in this group. An underlying explanation for the clinical chemistry modifications could not be found.
The mean testes weights for ♂ receiving 0.4 and 2.0 mg/kg bw/d were slightly but significantly decreased in comparison to control values. Mean pancreas weights for ♀ receiving 0.4 and 2.0 mg/kg bw/d were significantly greater than the control values, but in the absence of meaningful histopathological effects these findings are of uncertain toxicological relevance.
Periportal hepatocyte vacuolation and lipid deposition were found in the livers of control and treated dogs. However, both the incidence and severity of these findings were increased for animals receiving 0.4 and 2.0 mg/kg bw/d. A degree of thymic involution was observed for 2 animals receiving 0.4 mg/kg bw/day and 3 animals receiving 2.0 mg/kg bw/d.
The **NOAEL** is set at **0.4 mg/kg bw/d,** based on increased incidences/frequency of clinical signs (vomiting, excessive salivation), ↓bodyweight, haematological findings, single blood chemistry parameters, liver coarse texture, ↑thymic involution and ↑severity of lipid periportal depots and vacuolation at the top-dose LOAEL of **2 mg/kg b.w./d**.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Classification for STOT RE is warranted when repeated exposure to a substance results in 'significant' or 'severe' toxicity, generally at doses that are around or below the reference values assigned in the guidance on the application of the CLP criteria (ECHA-17-G-21-EN; 10.2823/124801).
In the context of classification:
'significant' is taken to mean morphological changes that are toxicologically significant, or effects that clearly indicate functional disturbance.
'Severe' refers to more profound effects of an adverse nature or effects, which significantly impact on health.

Metam (incl. -sodium and -potassium):

In the available repeated-dose toxicity studies effects on the haematopoietic system (red blood cell and clotting parameters), liver, kidney, bladder as well as local and general systemic effects (mortality, body weight) were observed. Notably, liver parameters were affected in mice and dogs after oral administration and in rats after inhalation. In the 90-day and 90-day neurotox rat drinking water studies no effects on the liver was observed. The lowest effect level was at 1 mg/kg bw/day in the dog 90-day study. The relevance of the effects at 1 mg/kg bw/day on the liver is questionable with regards to adversity. No microscopic correlates other than for control organs were observed. Moreover, no consistent or dose-related changes were observed and the values were generally covered by historical control data. The increase of mean ALT enzyme activities was due to measurements in one female animal. The increase of the ALT in this female animal was rather isolated as no consistent changes of ALP, AST or γ-GT enzyme activities were observed, which would have been observed in case of a severe liver damage. In the 90-day study in dogs increased liver enzymes were measured and hepatitis was determined microscopically at 5 mg/kg bw/day. Severe general systemic toxicity was observed at 10 mg/kg bw/d with the dogs showing decreased body weights and mortality occurring. However, in an additional study with 1 male and 1 female dog dosed with 10 mg/kg bw/day for 90 days with an additional 8 week recovery period, an intermittent increase of liver enzymes was observed which was reversible within the recovery period. No severe general toxicity was observed.

Metam sodium, orally administered via capsules to ♂ and ♀ Beagle dogs for 90 days, caused treatment-related hepatitis in both sexes dosed with 5 mg/kg bw/day and above, which resulted in the early termination of 2 dogs in the top dose group due to adverse clinical signs. The onset of this treatment-related hepatitis showed a clear dose and time relationship. Other modified parameters were marked increases in ALT, AST and ALP. The progressive inappetence and weight loss occurred later and coincided with increases in plasma γGT activity, total bilirubin and jaundice. Gross pathology revealed accentuated liver lobular pattern with pale appearance or depressed red areas. Marked hepatitis was seen microscopically. There was a variety of changes, which were considered secondary to the hepatotoxicity or indicative of generalised toxicity or poor clinical conditions. Taking into account the clinical signs, and both liver and kidney/urinary bladder adversity observed at the **LOAEL of 5 mg/kg bw/day** a NOAEL of 1 mg/kg bw/day was set.
This LOAEL is below the guidance value of **10 mg/kg bw/day** , justifying the proposal of classification of metam-sodium as specific target organ toxicant following repeated exposure (STOT-RE 1).

In conclusion, **STOT RE Cat. 1 classification for the target organ liver** is feasible when taking into account the dog studies.

MITC:
In the available repeated-dose oral toxicity studies no primary target of MITC could be identified neither in the rat nor in the mouse or dog. In the available repeated-dose toxicity studies, where MITC was applied via the inhalation route, most critical effects were restricted to the respiratory tract due to the corrosive properties of MITC. Since the substance is classified as corrosive, an irritation of the respiratory tract by the vapour could be expected and has been observed at 20-240 mg/m³ (corresponding to 0.02 to 0.24 mg/L). It is assumed that the irritation would increase with higher concentrations. The corrosive/irritation potential observed in repeated dose toxicity studies is mostly covered by the classification as 'corrosive' Category 1, and no additional classification as STOT-RE with respect to the inhalation route would result.
Nevertheless, in an acute inhalation study with MITC, mortality was observed at ≥496 mg/m³. According to the guidance on the application of the CLP criteria, classification for STOT RE could be considered if the dose leading to adverse effects is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity). In this case, classification as specific target organ toxicant (repeated exposure) would be warranted even if the substance is also classified as acutely toxic and/or corrosive. In the available studies critical effects were observed from 1.6 to 90 mg/m³ and thus being more than half an order of magnitude lower than the evident acute effect.
.
In Vol.3 B6, the assessment was expanded with a tabulated summary of the histopathological findings in lung and liver (see figure B.6.8.1.1/03-1, and tables B.6.8.1.1/03-4 (histopathology in decedents) and B.6.8.1.1/03-5 (histopathology in surviving animals).
The findings indicate that there is a close association between the very steep dose-dependent increase of mortality from 0.496 mg/L onwards (almost linear dose-response in the dose-fork 0.496-0.628 mg/L). It confirms that the **acute** effect of MITC on the lungs (lung congestion and associated lesions) are the cause of death.

In contrast, in a 90-day inhalation study with MITC ([redacted], 2013), severe upper respiratory tract **subchronic** effects (metaplasia of the squamous epithelium, single cell necrosis in the olfactory epithelium and the larynx, and atrophy of the glandular epithelium) was observed at about 5 ppm (equivalent to 15 mg/m³) onwards, *i.e.* below the guidance value of 50 ppm for gases, and in the absence of mortality.
Therefore, the substance qualifies for a classification according to the CLP: STOT-RE1, with H-statement H372 «Causes damage to the upper respiratory tract through prolonged or repeated exposure by inhalation».
This signifies that, in RMS opinion, the establishment and rationale for the STOT RE1 of MITC is justified.

In conclusion, classification as **STOT RE Cat. 1 based on effects on the respiratory tract** is proposed.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Metam (incl. -sodium and -potassium):

Classified – STOT RE, Category 1 (H372: Causes damage to organs (liver) through prolonged or repeated exposure)

MITC:

Classified – STOT RE, Category 1 (H372: Causes damage to organs (upper respiratory tract) through prolonged or repeated exposure)

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Table 50a: Summary of *in-vitro* genotoxicity of Metam sodium.

Type of test, Test method, GLP	Conditions (strains/ species, concentrations tested)	Metam sodium, B. n°, Purity (%)	Results, Conclusion	Reference, Study n°
Bacterial reverse mutation assay (Ames) OECD 471 (1981) GLP compliant	*S. typhimurium* strains TA 92, TA98, TA100, TA1535, TA1537, TA1538 Treat and plate:20 – 5000 µg/pl (±S9) Preincubation: 4 – 2500 µg/pl (±S9)	B n° ZH130585; Purity: 42.2%	A bacteriotoxic effect depending on the strain, experiment and test conditions from about 100-2500 µg/plate onward. No increase of the incidence of revertants. Conclusion: **negative**	[redacted] (1987a) **B.6.4.1.1/01**
Bacterial reverse mutation assay (Ames) OECD 471 (1997) GLP compliant	*S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strain WP2*uvrA* *Treat and plate:* • 0.71-5000 µg/pl (TA100, WP2*uvrA* (±S9) • 7.1-2083 µg/pl (TA1535, TA1537, TA98, ±S9) • 7.1-5000 µg/pl (TA1537, ±S9) *Preincubation:* 17-5000 µg/pl (+S9)	B. n° G180337730 Purity: 41.7%	No precipitation Bacteriotoxicity depending on strain, experiment and test conditions from about 417-2083 µg/plate onward, except in WP2uvrA +S9 mix where no toxicity was observed at any of the dose levels tested. No increase of the incidence of revertants. Conclusion: **negative**	[redacted] (2019a) **B.6.4.1.2/01**
Gene mutation (HPRT) in CHO cells OECD 476 (1984) GLP compliant	Chinese Hamster Ovary cells 0.0464 – 10 µg/mL (±S9)	B n° ZH130585; Purity: 42.2%	Cytotoxicity at top-concentration, 24h after exposure termination. Precipitation at 1000 µg/mL in the range finding study. Increase of mutation rate at the cytotoxic concentration of 10 µg/mL. • positive response in 1 of 3 experiments –S9 • positive response in 2 of 3 experiments +S9 The results were poorly concentration-related and the mutation rate of all negative controls was zero. Conclusion: **equivocal**.	[redacted] (1987b) **B.6.4.1.1/02**
Mammalian gene mutation (TK) in L5178Y mouse lymphoma cells OECD 490 (2016) GLP compliant	Mouse lymphoma L5178Y cells (TK locus) • 0.10-83 µg/mL (–S9) 0.21-125 µg/mL (+S9)	B. n° G180337730 Purity: 41.7% w/w	Cytotoxicity (↓RTG) at ≥31 µg/mL (–S9) and at ≥83 µg/mL (+S9) Concentrations at ≥31µg/mL in the genotoxicity assay are acceptable. • positive response at ≥31 mg/mL –S9 • positive response at ≥83mg/mL +S9 The results were concentration-related. Conclusion: **positive**	[redacted] (2019b) **B.6.4.1.2/02**

Type of test, Test method, GLP	Conditions (strains/ species, concentrations tested)	Metam sodium, B. n°, Purity (%)	Results, Conclusion	Reference, Study n°
***In-vitro* Chromosomal aberrations** in human lymphocytes OECD 473 (1983) GLP compliant	Human lymphocytes, 24h sampling time • 1 – 20 µg/mL (24h –S9) • 10 – 40 µg/mL (2h +S9) Dose selection based on a preliminary cytotoxicity assay (1 –40 µg/mL)	B n° ZH130585; Purity: 42.2%	Cytotoxicity at ≥20 µg/mL (–S9) No cytotoxicity up to and including 40 µg/mL +S9 ↑M.I. ≥5 mg/mL –S9 and at ≥20 µg/mL +S9 Concentrations at ≥10 µg/mL in the genotoxicity assay are acceptable. • positive response at ≥10 mg/mL –S9 (stat. sign. at 20 µg/mL) • positive response at ≥10 mg/mL +S9 (stat. sign. at ≥20 µg/mL) The results were concentration-related; chromosome exchanges at 20 µg/mL (±S9) and above (+S9). Conclusion: **positive**	[redacted] (1987a) **B.6.4.1.1/03**
***In-vitro* Chromosomal aberrations** in human lymphocytes OECD 473 (1983) GLP compliant	Human lymphocytes, 72h sampling time • 2.5 – 30 µg/mL (24h –S9) • 5 – 40 µg/mL (2h +S9) Dose selection based on a preliminary cytotoxicity assay (2.5–40 µg/mL)	B n° WCR 14989-13-01; Purity: 32.3%	Cytotoxicity (↓M.I.) at 30 µg/mL (–S9) and ≥20 µg/mL +S9 Concentrations at ≥5 µg/mL in the genotoxicity assay are acceptable. • negative response at 30 mg/mL –S9 • positive response at ≥20 mg/mL +S9 (stat. sign. at ≥20 µg/mL) The results were concentration-related. Conclusion: weakly **positive**	[redacted] (1996a) **B.6.4.1.1/04**
***In-vitro* UDS in rat hepatocytes** OECD 482 (1986) GLP compliant	Primary ♂ Fischer 344 rat hepatocytes 0.5 – 250 nL/mL	B. n° ZH130585; Purity: 42.2%	Metam sodium did not induce significant changes in the nuclear labelling of hepatocytes. Conclusion: **negative**	[redacted] (1987) **B.6.4.1.1/05**
Limited acceptability or not previously accepted				
Bacterial assay for DNA damage – (Rec-assay) No OECD guidance GLP compliant	*B. subtilis* strains H17 (Rec^+) and M45 (Rec^-) • 1st and 2nd exp.: 0.05 – 76.5 µg/pl, ±S9 • 3rd exp.: 0.05 – 76.5 µg/plate, ±S9	B. n°: ZH 130585 Purity: 42.2%	No precipitation Increase of the difference of zones of inhibition of the strains M45 and H17 was not observed for any test substance concentration (± S9) Conclusion: **negative**	[redacted] (1987) **B.6.4.1.1/06**
Bacterial reverse mutation assay (Ames) Equivalent to OECD 471 (1981) Not GLP compliant	*Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 0-1600 µg/plate (± S9)	B n° and purity: not reported	No precipitation observed Bacteriotoxicity –S9 from at ≥160 µg/plate No increase of the incidence of revertants. Conclusion: **negative**	[redacted](1979) **B.6.4.1.1/07**

Table 50b: Summary of *in vitro* genotoxicity of MITC

Type of test **Cell/Test species, guidelines, GLP**	**Conditions : test organism, concentrations/doses tested ; route of administration, sampling time**	**MITC** **Batch n°, purity (%)**	**Results**	**References**
Bacterial reverse mutation assay (AMES) OECD 471 (1997) Not GLP compliant Study supplemental.	*S. typhimurium* TA98, TA100, TA1535, TA1537 Concentrations tested: 20, 100, 500, 2500 and 5000 µg/plate (test I) and 30, 60, 125, 250 and 500 µg/plate (test II) (±S9)	B. n° 6205 MK 98.1%	**Negative** No precipitation. Cytotoxic effects depending on the strain and study at doses ≥ 500 µg/plate.	[redacted] 1986 **B.6.8.1.3.1/01**
Bacterial reverse mutation assay (AMES) OECD No. 471 (1997) GLP compliant Study accepted.	*S. typhimurium* TA1535, TA1537, TA98, TA100 ; *E. coli* WP2*uvrA* Concentrations tested: 0.158-500 µg/plate (0.0016-1.6 mg/mL) (±S9)	B. n° STBB1308V 99.6%	**Negative** No precipitation Cytotoxic effects depending on the strain and study at doses ≥160 µg/plate.	[redacted] 2019 **B.6.8.1.3.1/04**
Bacterial assay for DNA damage – (Rec-assay) Guideline not available GLP compliant Study accepted.	*B. subtilis* H17 (rec^+) - M45 (rec^-) Concentrations tested: 1.0, 10.0, 100, 500, 1001, 2502, 5004, 10007 µg/plate (±S9)	B. n°: 6205 MK 98.1%	**Negative** No precipitation No cytotoxic effects	[redacted] 1989 **B.6.8.1.3.1/03**
Mammalian cell gene mutation Test OECD No. 490 (2015) GLP compliant Study accepted.	Mouse lymphoma cells (L5178Y $TK^{+/-}$) Concentrations tested: 0.50, 0.74, 1.1, 1.7, 2.5, 3.8 µg/mL (+S9) 0.13, 0.20, 0.30, 0.44, 0.67, 1.0, 1.5 µg/mL (–S9)	B. n°: STBB1308V 99.6%	**Positive (+S9)** dose-dependent ↑MF and ↓RTG from 82 to 2%, at 2.5-3.8 µg/mL. **Equivocal (–S9)** ↑MF and ↓RTG from 98 to 7%, at 1.0-1.5 µg/mL Conclusion: positive +S9 and equivocal –S9 at concentrations including highly cytotoxic doses.	[redacted] 2020 **B.6.8.1.3.1/06**
Mammalian chromosome aberration assay (CA) OECD 473 (1983) GLP compliant Study accepted.	Human peripheral blood lymphocytes Concentrations tested: 0.05, 0.1, 0.5 µg/mL (–S9) 0.1, 0.5, 1.0 µg/mL (+S9)	B. n° 6205 MK 98.1%	**Negative** No dose-dependent nor statistically significant ↑cells with structural CA excluding gaps. ↑structural CA including gaps. No ↑numerical aberrations.	[redacted], 1987 **B.6.8.1.3.1/02**
***in vitro* micronucleus test** OECD No. 487 (2016) GLP compliant Study accepted.	Chinese Hamster Ovary (CHO) cells Concentrations tested 0.078 – 2.0 µg/mL **(–S9** tested **only)**	B. n°: STBB1308V 99.6%	**Positive** Dose-dependent ↑MN (micronuclei) and ↑HD (hypodiploid) in cells at conditions of no (0.3958 µg/mL, RICC 114%) to slight (0.59 µg/mL, RICC 78%) up to high (2.0 µg/mL, RICC 2%) cytotoxicity.	[redacted] 2020 **B.6.8.1.3.1/05**

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells ***in vivo***

Table 51a: Summary of *in-vivo* genotoxicity of Metam sodium.

Type of test, Test method, GLP	Conditions (strains/ species, doses tested)	Metam sodium, B. n°, Purity (%)	Results, Conclusion	Reference
***In-vivo* micronucleus assay** (mouse bone marrow) OECD 474 (1983) GLP compliant	Mouse (CD-1) ♂ and ♀ (5/sex/group) **500 mg/kg bw** (dosing volume 10 mL/kg bw) by gavage BM sampling 24 and 48 h after dosing (dose selection based on preliminary toxicity study)	B n° WCR 14989-13-01 Purity : 32.3%	No relevant ↑ in the number of micronucleated PCE and no ↑ in the % PCE in either sex. The presence of a.s. or its metabolites in BM or blood plasma not assessed. Conclusion: **negative**, target organ BM not demonstratively attained.	[redacted] (1996) **B.6.4.2.1/01**
***In vivo* chromosomal aberration assay** (Chinese Hamsters bone marrow) OECD 475 (1984) GLP compliant	Chinese hamsters ♂ and ♀ (10/sex/group) **0, 150, 300 and 600 mg/kg bw** (dosing volume 10 mL/kg bw) by gavage BM sampling 6, 24 and 48 h after dosing	B. n° ZH130585 Purity: 42.2%	Doses of the test substance led to irregular respiration about 30' after the administration for about 1-2 hours. No ↑ in the number of aberrant metaphases at 6h or 48h after treatment, but statistically significant ↑ at 24h. No ↑ in the type and frequency of aberrations between the dose groups and the solvent control group. Conclusion: **equivocal**	[redacted], (1987b) **B.6.4.2.1/02**
Combined*:* ***In vivo* micronucleus assay** (rat bone marrow) ***In vivo* comet assay** (rat liver and stomach cells) OECD 474 (2016) OECD 489 (2016) GLP compliant	Wistar Han ♂ (5 /group) **0, 87.5, 175, 350 mg/kg bw** by gavage (once daily for 3 consecutive days) Bone marrow and liver/stomach cell sampling 3-4 h after last dose (d3). (dose selection based on preliminary toxicity study - RF)	B. n°: G180337730 Purity: 41.7%	***Micronucleus assay:*** RF: mortality at 750 (♂,♀) and at 500 mg/kg bw (♀) on d2 or d3, none at 350 mg/kg bw. Main test: clinical signs but b.w. unaltered at any dose. Marginal non stat. sign. ↓PCE/NCE at the top-dose (~18%). The presence of a.s. or its metabolites in BM or blood plasma not assessed. Conclusion: *in-vivo* micronucleus test: **negative**, target organ BM not demonstratively attained. ***Comet assay:*** Dose-dependent increase of tail intensity of the comets in stomach cells, and stat. sign. increase in both liver and stomach cells at top-dose. Evidence of toxicity at top-dose (lower doses not assessed). Whether the comet finding reflects a direct genotoxicity or is a consequence of cytotoxicity is unclear. Conclusion: *in-vivo* comet assay: **positive.**	[redacted] (2020), **B.6.4.2.2/01**

Table 51b: Summary of *in vivo* genotoxicity of MITC

Type of test Cell/Test species, guidelines, GLP	**Conditions : test organism, concentrations/doses tested ; route of administration, sampling time**	**MITC Batch n°, purity (%)**	**Results and discussion**	**Reference, Study n°**
Micronucleus test in mouse bone marrow. OECD No. 474 (1983) GLP compliant Study accepted.	Mouse (CD-1) Single oral dose (gavage); a.s. dissolved in corn oil, ♂/♀, main test 110 mg/kg bw (5 mice/sex/time point). Bone marrow sampling 24h, 48h, 72 h after dosing. Dose selected in range-finding (RF) tests: 1st RF 20, 40, 40, 80, 160, 320 mg/kg bw (2 mice/sex) 2nd RF 80, 100, 125, 156.25 mg/kg bw (5 mice/sex)	B. n°: 340178 B19 (RF test) 340178 B23 (main test) 95.86%	**Negative** No evidence of MN induction at a toxic dose of 110 mg/kg b.w., inducing ↓PCE/NCE at 24h and 48h, indicating that BM was reached.	[redacted] 1985 **B.6.8.1.3.2/01**
Micronucleus test in mouse bone marrow. OECD No. 474 (1997) GLP compliant Study accepted.	Mouse (CD-1) Single 6-hour inhalation exposure to ♂/♀ mice (5-6 mice/sex/time point). target concentration: 0, 20, 40 and 100 ppm actual concentrations: 0, 20, 40 and 98 ppm equivalent to 0, 60, 120 and 294 mg/m³ Bone marrow sampling 24h, 48h after dosing.	Batch n°: 56198PJV 99.7%	**Negative** No evidence of MN induction at a toxic dose of up to 300 mg/m³ (~80 mg/kg b.w.), only slight ↓PCE/NCE (48h), no MITC dosage in blood. WoE that BM was reached (PCE/NCE changes overall, BM alterations in LT assays, rat ADME study, observed toxicity at the tested doses, presence of [^{14}C]MITC in BM cells).	[redacted] 2011, 2020 **B.6.8.1.3.2/02** **B.6.8.1.3.2/03**
Micronucleus test in rat bone marrow. OECD No. 474 (2016) GLP compliant Study accepted.	Rat Crl:CD (SD) Oral dose (gavage, 2×at 21h interval); a.s. dissolved in corn oil, (5-6 ♂rats/time point). Main test:0, 20([1]), 40([2]) and 60([1]) mg/kg bw *([2]:only mid-dose radioactively labelled, purities different)* Dose selected in range-finding (RF) see [redacted], 2020. Bone marrow sampling 24h after dosing. oral gavage, 20, 40 and 60 mg/kg bw doses for two consecutive days 24 hours apart, 5 ♂/dose (but 6 ♂ for top-dose).	B n°([1]): 7JVGB 98.1% **[^{14}C]MITC** B. n°([2]): CP-4443 96.51%	**Negative** No evidence of MN induction up to and including the top-dose 60 mg/kg bw, where adversity is observed. No ↓PCE/NCE (48h), but **[^{14}C]MITC** dosage in BM (40 mg/kg b.w); target tissue considered reached No toxic effects in control and 20 mg/kg bw. Dose-dependent ↓b.w.g. at ≥40 mg/kg bw and clinical signs at top-dose. *Note: based on the assay set-up (different MITC sources used), the results of the MNPCE frequency at 40 mg/kg bw/day are not entirely comparable with those of the other dose-groups.*	[redacted] 2020 **B.6.8.1.3.2/05**

***In-vivo* comet assay in rats** OECD No. 489 (2016) GLP compliant Study accepted.	Rat Crl:CD (SD) Oral dose (gavage, 2× at 21h interval); a.s. dissolved in corn oil, (5 ♂rats/time point). Main test:0, 20, 40 and 60 mg/kg bw Dose selected in range-finding (RF) test in ♂/♀: 0, 20, 40, 60 and 80 mg/kg bw doses twice at 21 hour interval (3 rats/sex/dose). Liver and stomach tissue sampling 24h after dosing.	Batch n°: 7JVGB; 98.1%	**Negative** No evidence of DNA damage in neither liver nor stomach cells 24h after the dosing Clinical signs and b.w. effects, histopathological findings at 60 mg/kg bw in both liver (hepatocellular focal necrosis and centrilobular vacuolation) and stomach (submucosal oedema and erosion/ulcer). No meaningful toxic effects in control, 20 and 40 mg/kg bw.	[redacted], 2020 **B.6.8.1.3.2/04**

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam				
No data available				
MITC				
No data available				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

Metam (incl. -sodium and -potassium):

In the previous dossier, an Ames test ([redacted], 1987a, **B.6.4.1.1/01**), an HPRT assay in CHO cells ([redacted], 1987b, **B.6.4.1.1/02**), two chromosome aberration tests ([redacted], 1987, **B.6.4.1.1/03** and [redacted], 1996a, **B.6.4.1.1/04**), an unscheduled DNA synthesis (UDS) assay ([redacted], 1996, **B.6.4.1.1/05**), a Rec-Assay ([redacted], 1987, **B.6.4.1.1/06**) and an old, non-accepted Ames test ([redacted], 1979, **B.6.4.1.1/07**) were provided in the *in vitro* section. New *in vitro* studies, an Ames test ([redacted] 2019a, **B.6.4.1.2/01**) and a Mouse Lymphoma Assay ([redacted], 2019b, **B.6.4.1.2/02**) were performed according to the current OECD test guidelines.
Regarding *in vivo* studies, one mouse micronucleus (Barber-Mackay, 1996, **B.6.4.2.1/01**) study and one chromosomal aberration test ([redacted], 1987b, **B.6.4.2.1/02**) were detailed in the previous dossier. A comet assay with integrated bone marrow sampling for assessment of micronucleus, performed *in vivo*, was recently provided ([redacted] 2020, **B.6.4.2.2/01**).

***In vitro* studies**

In the bacterial revertant assay performed by [redacted] (1987a, **B.6.4.1.1/01**), employing the standard plate test as well as the pre-incubation test method metam sodium was **negative** both in the presence and absence of metabolic activation towards *S.typhimurium* strains TA92, TA98, TA100, TA1535, TA1537 and TA1538. This was confirmed by a more recent bacterial test using *Salmonella* and *E. coli* strains, where no mutagenic effect was observed ([redacted], 2019, **B.6.4.1.2/01**).

In the HPRT assay in CHO cells ([redacted], 1987b, **B.6.4.1.1/02**), slight increases in mutation rates cannot be ruled out completely although the results are not dose-related. A positive response in 2 out of 3 experiments in the presence of metabolic activation and in 1 out of 3 experiments in the absence of metabolic activation, cannot entirely be discounted (albeit with a zero incidence in all controls, and showing poor concentration-dependency in most cases) and is therefore considered **equivocal**.

In the Mouse Lymphoma Assay - MLA ([redacted] 2019, **B.6.4.1.2/02**), the test item induced dose-related increases in the mutation frequency in the main experiment (±S9). The notifier concludes that "*the results of this MLA should not be used in isolation for the final classification of metam for genotoxic properties*". **RMS** considers that based on the experimental results, metam sodium increases the mutant frequency both in the absence and in the presence of metabolic activation. The positive result occurs above the control+GEF incidence at ≥31 µg/mL and at ≥83 µg/mL, in the absence and in the presence of metabolic activation, respectively. Overall, a trend for a **positive** response is visible at 21 µg/mL and above.

Two *in-vitro* chromosome aberration assays were performed on human lymphocytes.
Metam sodium revealed clastogenic activity both in the presence and absence of metabolic activation in the first assay ([redacted], 1987a, **B.6.4.1.1/03**). The original study report mentions no particular cytotoxicity at the doses where clastogenicity occurred, although a re-examination in 1996 concluded on *«severe toxic effects on the chromosome morphology»*. However, in the absence of details, or without a valid indication by which MoA the chromosomic structure would refer to any (a)specific toxicity, **RMS** cannot fully take into consideration this statement.
RMS concludes that Metam sodium has a clastogenic effect *in vitro* using human lymphocytes, both in the absence and in the presence of metabolic activation, and the a.s. should thus be considered **positive** under the current experimental conditions.
In the second test ([redacted] 1996a, **B.6.4.1.1/04**), **RMS** considers that, although the increase in aberrant cells is low, the study produced slight positive results in the presence of metabolic activation, and should be considered as **weakly positive**, at doses where *no* excessive cytotoxicity was noted (↓40% M.I. *vs.* controls).

In the *in vitro* UDS assay to assess DNA damage in freshly isolated primary rat hepatocytes ([redacted], 1996, **B.6.4.1.1/05**), metam sodium did not induce unscheduled DNA synthesis even at concentration being cytotoxic as demonstrated by the net nuclear grain count and the percentage of nuclei with more than 6 or 20 grains.

***In vivo* studies.**
An *in vivo* mouse micronucleus assay ([redacted], 1996, **B.6.4.2.1/01**) was conducted *via* gavage (at the MTD of 500 mg/kg bw). No increase in the number of micronucleated polychromatic erythrocytes (PCE) was observed, but no meaningful change in the percentage of PCE were observed in either sex. It is of note that exposure of Metam in the bone marrow exposure was not verified by blood analysis.

In an *in vivo* chromosomal aberration test on bone marrow cells of Chinese Hamsters ([redacted], 1987b, **B.6.4.2.1/02**), the test outcome was concluded to be **equivocal** for clastogenicity. The incidence of cells showing clastogenicity was statistically significantly increased in the top-dose animals sacrificed at 24h after the treatment, but no increases were seen at neither 6h nor 48h after administration. Therefore, it is thought that the increase is of uncertain genotoxicological relevance. Involvement of the treatment is not completely excluded taking into account the equivocal/weak result in some *in-vitro* assays.

With regard to the *in-vivo* micronucleus test ([redacted], 2020, **B.6.4.2.2/01**) the weight of evidence indicates a systemic exposure to metam-sodium referring to the observations in the study and general available data for metam. However, in this experiment, plasma analysis to demonstrate bone marrow exposure could not be performed, because of the quick breakdown of the a.s.. Mortality was observed in the dose range finder at 500 and 750 mg/kg bw. Clinical signs of toxicity were observed in all animals treated with metam. The PCE/NCE ratio was reduced by 18% (11% to 25% for single animals) although not statistically significant. ADME studies in rat indicated that metam is rather completely absorbed and excreted via urine, but these studies did not demonstrate the presence of neither the the a.s. nor its metabolites in the bone marrow. Clinical signs and target organ effects, which were observed suggest comparable bioavailability within rodent species, but in neither species, bone marrow was demonstrated to have been in contact with metam-sodium.
In the *in-vivo* comet assay performed in the same experiment, increases of tail intensities were observed at the top-dose of 350 mg/kg b.w. in both liver and stomach tissue. Some organ toxicity was observed in liver (hyperplasia, necrosis) and stomach (erosion/ulcer, oedema, inflammation) in all animals at the top dose.
According to **RMS**, whether these effects reflect a direct genotoxicity or are a consequence of cytotoxicity remains unclear.

Additional studies

In an *in vitro* Rec-assay ([redacted], 1987, **B.6.4.1.1/06**) that was considered of limited acceptability, due to the inconsistent positive control responses, metam sodium was not recombinogenic to *Bacillus subtilis* indicator organism.

The Ames test from [redacted] (1979, **B.6.4.1.1/07**) was not relied upon in the DAR (revised 2010). For the convenience of the reviewer and for the sake of completeness this study is nevertheless summarised. The results of this study were in line with the results of the studies relied upon.

In short, metam sodium genotoxicity is demonstrated *in vitro* and suggested in some *in-vivo* tests.

MITC:

A full set of genotoxicity studies (has been provided for the Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. Further studies with MITC have been performed >2010, as since the Annex I inclusion for metam the data requirements have been adapted a re-evaluation of the studies was performed.
These additional studies, performed according to the current OECD test guidelines, are an Ames test, a mouse lymphoma assay, an *in-vitro* and two *in-vivo* micronucleus assays (each in the mouse and in the rat), with a statement on plasma/bone marrow exposure of MITC, and an *in-vivo* comet assay in rats. In total, following studies were performed:

- *in vitro* in two Ames *S. typhimurium* reverse mutagenesis assays ([redacted] 1986 -**B.6.8.1.3.1/01**; [redacted] 2019 – **B.6.8.1.3.1/04**), one recombination bacterial assay for DNA damage (Rec-assay, [redacted] 1989 – **B.6.8.1.3/03**), one chromosome aberration study using human lymphocytes ([redacted], 1987, - **B.6.8.1.3.1/02**), one micronucleus test using CHO cells ([redacted], 2020 – **B.6.8.1.3.1/05**), and one mouse lymphoma assay ([redacted], 2020 – **B.6.8.1.3.1/06**);
- *in vivo* in two mouse micronucleus assays ([redacted], 1985, - **B.6.8.1.3.2/01**- ; [redacted], 2011, **B.6.8.1.3.2/02**), with a statement on plasma/bone marrow exposure – [redacted] 2020 **B.6.8.1.3.2/03**), one rat comet assay ([redacted] 2020, -**B.6.8.1.3.2/04**) and one rat micronucleus assay ([redacted], 2020, - **B.6.8.1.3.2/05**).

***in vitro* studies**
The Ames bacterial mutagenicity ([redacted] 1986 - **B.6.8.1.3.1/01**; [redacted], 2019 - **B.6.8.1.3.1/04**) and recombination assay ([redacted], 1989 - **B.6.8.1.3/03**) studies were negative both with and without S9 enzymatic activating system.

In the *in vitro* chromosome aberration study ([redacted] 1987 - **B.6.8.1.3.1/02**), there was a statistically significant increase in the incidence of chromosomal aberrations (including gaps only), both in the presence and the absence of S9 enzymatic activating system. There was no increase in the incidence of polyploid metaphases in any

experimental conditions in that study. Overall, the outcome of this study is negative.

MITC is capable of inducing mutations in the mammalian cell gene mutation (mouse lymphoma assay) test ([redacted], 2020 - **B.6.8.1.3.1/06**) in cultured L5178Y $TK^{+/-}$ 3.7.2C cells when tested up to the limit of toxicity or at high cytotoxicity, in the presence of S9 metabolic activation system, while the outcome remains equivocal in the absence of S9 metabolic activation system. The positive results were related to dose-dependent increases of both large colonies as well as small colonies, indicating an involvement of both gene-mutations and chromosome aberrations, respectively. An increase of all colonies exceeded the GEF only at cytotoxic doses ≤25% (–S9) or ≤16% (+S9) RTG of controls.

In the *in vitro* micronucleus test in CHO cells ([redacted], 2020 – **B.6.8.1.3.1/05**) a dose-dependent increased rate of micronuclei was observed after 4 hours in the absence of S9, in conditions of no to strong cytotoxicity. For technical reasons, 4h +S9 and 26h –S9 were not assayed.

***in vivo* studies**

Two *in vivo* mouse micronucleus assays were conducted via oral (110 mg/kg bw *p.o.*, [redacted], 1985 - **B.6.8.1.3.2/01**) and inhalation route (60, 120 and 294 mg/m³ *p.i.*, [redacted], 2011 - **B.6.8.1.3.2/02**). Clinical signs of toxicity were observed for both studies, such as lethargy, ptosis or respiratory rate (p.o.) or body weight loss, decreased body weight gains and lower food consumption (p.i.). However, there was no effect on the incidence of micronucleated polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes, except for p.o administration (decrease in PCE/NCE ratio in animals sacrificed 48h post dosing).

In another recent rat micronucleus assay ([redacted] 2020, - **B.6.8.1.3.2/05**), there was evidence that bone marrow cells of the rats in this micronucleus test were exposed to radioactivity after [^{14}C]MITC oral administration. The decrease in MNPCE frequencies, statistically significant but for which a dose-response was not seen, remained unexplained. **RMS** note: Based on the assay set-up (different MITC sources, and thus purities tested), the RMS expressed some doubt on the value of the MNPCE frequency at 40 mg/kg bw/day, but overall the study is not invalidated.

The notifier developed an argumentation regarding the systemic availability of MITC in the *in-vivo* genotoxicity studies ([redacted], 2020, **B.6.8.1.3.2/03**). Several lines of evidence were highlighted in order to demonstrate that the bone marrow could likely be reached subsequent to exposure to MITC. The rationale, based upon the data in the toxicity guideline studies included:

(i) toxicity to the bone marrow observed in the mammalian erythrocyte micronucleus test: decrease of PCE/TE (total erythrocyte) proportion at 48h sacrifice time, although this was not seen in all studies;

(ii) toxicity to the bone marrow observed in toxicity studies, especially in the rat and mouse chronic toxicity studies (bone marrow hyperplasia and single incidences of bone marrow necrosis, small round cell infiltration),

(iii) MITC (and/or metabolites) detected in the bone marrow in the rat toxicokinetic study and presence of radioactively labelled [^{14}C] MITC in mouse bone marrow, and finally,

(iv) toxicity observed in toxicity studies, including the bone marrow micronucleus test.

On this basis, it seems plausible for the **RMS** that there was systemic exposure to MITC to the bone marrow, when administered orally or via inhalation.

In a recent preliminary comet assay in rats ([redacted], 2020, -**B.6.8.1.3.2/04**), using 20, 40, 60 or 80 mg/kg bw/day of MITC a double dose of 60 mg/kg bw/d was identified as MTD for double treatment in ♂ and ♀ rats. In this preliminary study, no significant DNA damage, as expressed by an increase in % tail DNA, was seen for liver and stomach.

Also in the full study, using 20, 40 and 60 mg /kg bw/day MITC, no DNA damage was evident in neither the liver nor stomach of ♂ rats.

It was concluded that MITC did not induce DNA damage in these tissues in an acute toxicity dosage.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

Metam sodium

Based on the collected data, the RMS concludes that Metam sodium genotoxicity is demonstrated *in vitro* and suggested in some *in-vivo* tests.

Some **positive** responses were observed in *in-vitro* assays (mammalian gene-mutation and clastogenicity in 1 out of 2 assays each). No clear clastogenicity was observed *in-vivo*,(only 1 equivocal clastogenic outcome out of 3

assays) but it could in no case be unequivocally demonstrated that the target organ (bone marrow) was attained, and in these studies, the presence of neither a.s. nor its metabolites in bone marrow or blood was demonstrated.

A **positive** outcome in an *in-vivo* comet assay (both in stomach and liver), was observed. The MoA for the genotoxicological findings (either corrosivity, toxicity and/or GSH depletion associated to oxidative stress) is plausible, and it is of note that the comet assay is an indicative test only for genotoxicity, but not conclusive overall. In addition, the potential implication of the various degradates of metam (MITC, MIC, CS_2, COS, methylamine) remains unclear as well.

It is unclear whether metam sodium is genotoxic or not in *in vivo* systems. Its effects could be a consequence of cytotoxicity, as suggested but not definitely proved by the comet assay on liver and stomach. According to the notifier, "*effects on gastric tissue are considered due to the corrosive properties of metam in combination with the bolus application. Effects in the liver are considered due to the GSH-depleting properties of metam and were consistently observed in available short- and long-term studies. In conclusion, the corrosive and GSH-depleting properties of metam can explain the effects consistently observed in vivo at highest doses, affecting tissues with high local exposure or tissues depending on GSH-levels or general metabolic homeostasis as liver and olfactory epithelium (Bowman´s and Steno´s glands)*".

Overall, **RMS** is not entirely convinced that such MoA and associated general toxicity would be a sufficient reason to disregard completely the genotoxicological relevance of metam-sodium:

- classification in **Category 1B** may be based on positive results of at least one valid *in vivo* mammalian germ cell mutagenicity test. In case there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied. No positive germ cell assay is present, and in the context of debatable results in somatic cells (see below), Category 1B is obviously not applicable.

- classification in **Category 2** may be based on positive results of at least one valid *in vivo* mammalian somatic cell mutagenicity test, indicating mutagenic effects. A Category 2 mutagen classification may also be based on positive results of a least one valid *in vivo* mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results. RMS notes that with metam-sodium, there is a situation with a mixed outcome, showing both positive and negative results, which, according the CLP guidance, might still lead to classification.

Most tests detecting a certain type of mutation (e.g. point mutations) have been positive and all tests detecting chromosome mutations have been negative. Such circumstances clearly warrant classification although several tests have been negative which is plausible in this case.

The evaluation of genotoxicity assays on metam-sodium, detailed in vol.3 B.6, more specifically tables B.6.4.1a and B.6.4.1b offers a comprehensive overview illustrating the above evaluation. In short, it further summarises as follows:

Metam-sodium

Type of test	**Conclusion**	**Reference, Study n°**
***in-vitro* genotoxicity**		
Bacterial reverse mutation assay (Ames)	**negative**	[redacted] (1987a), **B.6.4.1.1/01**
Bacterial reverse mutation assay (Ames)	**negative**	[redacted](2019a), **B.6.4.1.2/01**
Mammalian gene mutation (HPRT) in CHO cells	**equivocal**	[redacted](1987b), **B.6.4.1.1/02**
Mammalian gene mutation (TK) in L5178Y mouse lymphoma cells	**positive**	[redacted] (2019b), **B.6.4.1.2/02**
In-vitro Chromosomal aberrations	**positive**	[redacted] (1987a), **B.6.4.1.1/03**
In-vitro Chromosomal aberrations	weakly **positive**	[redacted] (1996a), **B.6.4.1.1/04**
In-vitro UDS in rat hepatocytes	**negative**	[redacted] (1987), **B.6.4.1.1/05**
***in-vivo* genotoxicity**		
In-vivo micronucleus assay	**negative**, target organ BM not demonstratively attained.	[redacted] (1996), **B.6.4.2.1/01**
In vivo chromosomal aberration assay	**equivocal**	[redacted], (1987b), **B.6.4.2.1/02**
Combined*:* *In vivo* micronucleus assay (rat bone marrow) *In vivo* comet assay (rat liver and stomach cells)	***Micronucleus assay:*** **negative**, target organ BM not demonstratively attained. ***Comet assay:*** **positive**	[redacted] (2020), **B.6.4.2.2/01**

RMS concludes therefore that, while the criteria would theoretically point to a possible genotoxic outcome of metam-sodium, since « *at least one valid in vivo mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results*» is present, the WoE also indicates that the picture is not completely clear as a mixed response is produced, often in the presence of cytotoxicity. The limitations of the *in-vivo* studies are that a convincing evidence of target organ toxicity (notably in the bone marrow) is not provided, although it may be suspected that systemic toxicity is present.

-*The criteria further mention that in vitro* results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship (SAR) to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

In this case, there is no known SAR among dithiocarbamates like metam, pointing to known germ-cell mutagenicity. Rather, a.s. like thiram (see DAR) displayed a similar mixed outcome, where the expert consultation concluded that no classification for genotoxicity was warranted.

-Finally, there is no evidence of genetic damage to somatic cells in exposed humans shown to be caused by exposure to metam sodium, thus providing also no indication warranting classification as a Category 2 mutagen.

In the DAR Vol.3, RMS highlights that it is unclear whether metam sodium is genotoxic or not in *in vivo* systems. Its effects could be a consequence of cytotoxicity, as suggested but not definitely proved by the comet assay on liver and stomach. According to the notifier, *«effects on gastric tissue are considered due to the corrosive properties of metam in combination with the bolus application. Effects in the liver are considered due to the GSH-depleting properties of metam and were consistently observed in available short- and long-term studies. In conclusion, the corrosive and GSH-depleting properties of metam can explain the effects consistently observed in vivo at highest doses, affecting tissues with high local exposure or tissues depending on GSH-levels or general metabolic homeostasis as liver and olfactory epithelium (Bowman´s and Steno´s glands)».*

Overall, RMS is not entirely convinced that such MoA and associated general toxicity would be a sufficient reason to disregard completely the genotoxicological relevance of metam-sodium. On the other hand, RMS wishes to highlight that the main metabolite MITC, even less indicate a genotoxicity potential, although the metabolite is also displaying GSH-depleting characteristics. The latter may signify that GSH-depletion is either no valid MoA for genotoxicity, but otherwise leaves a potential genotoxic action of metam-sodium without plausible MoA.

No germ-cell assays are submitted, thus **RMS** is of the opinion that a possible classification for genotoxicity (**Muta 2**, H341, "*Suspected of causing genetic defects*") cannot be excluded.

RMS made an o**verview data and comparison with criteria for the establishment of genotoxicity C&L for metam-sodium.**

Category 1:
Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.
Substances known to induce heritable mutations in the germ cells of humans.
*The classification in **Category 1A** is based on positive evidence from human epidemiological studies.*
Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
This is obviously not the case for neither metam nor its main metabolite MITC.

*The classification in **Category 1B** is based on:*
– positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals;
This is obviously not the case for neither metam nor its main metabolite MITC.

or
– positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells;
This is obviously not the case for neither metam nor its main metabolite MITC.
Or

– positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
This is obviously not the case for neither metam nor its main metabolite MITC.

CATEGORY 2: Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.

*The classification in **Category 2** is based on:*
– Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
– Somatic cell mutagenicity tests in vivo, in mammals;
The two existing *in-vivo* genotoxicity assays (bone marrow micronucleus tests are negative ([redacted], 1996 and [redacted] 2020) although the target organ (BM) has not been convincingly reached. One *in-vivo* chromosome aberration assay ([redacted] 1987b) is equivocal.
There is no positive mammalian *in-vivo* somatic cell mutagenicity tests which is positive, but one *in-vivo* comet assay, which is merely testing DNA damage (genotoxicity).

Or

– Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.
The *in-vitro* mutagenicity assays display a mixed outcome, with some positive (*in-vitro* chromosome aberration, [redacted] 2019b, and mammalian gene mutation, [redacted], 1987a), some weakly positive (*in-vitro* chromosomal aberration, [redacted], 1996a), equivocal (mammalian gene mutation assay, [redacted], 1987) or negative (bacterial gene mutation assays ([redacted] 1987a and [redacted], 2019a).

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.
In the case of both metam and MITC, there is no evidence of molecular structural alerts for mutagenicity.

As suggested here above and further discussed below, the genotoxicity data package of metam indicated that, while there are some indications of interactions of metam with the genetic material, the criteria for classification of this a.s. as Muta Category 2 are not fully met. The guidance further states that a complex data situation with positive and negative results might still lead to classification. This is because all tests detecting a certain type of mutation (*e.g.* point mutations) have been positive and all tests detecting chromosome mutations have been negative. Such circumstances clearly warrant classification although several tests have been negative which is plausible in this case. In the case of metam, not all gene-mutation tests are positive, but only those targeting mammalian genes (thus not in the bacterial assays). **RMS** notes that a positive mammalian gene mutation assay in the presence of clearly negative bacterial gene mutation assays could possibly reflect «false positive» results, especially in the case when the test article would cause severe cytotoxicity. This could be the case with metam, in which case this would constitute an important argument for no classification instead of classification as Muta 2.

MITC
Based on the collected data, the RMS concludes that some MITC genotoxicity is shown *in vitro* but not *in vivo*.

Regarding MITC genotoxicity, it has been observed (see Kassie et al., 2001, **B.6.8.1.3.3/01**) that MITC induces only marginal effects at extremely high (almost lethal) doses in inner organs *in vivo*, but it causes DNA-damage at low concentrations *in vitro*. According to the authors, it is "*unlikely that exposure to a small amounts of MITC (i.e. a few milligram per person per day) causes DNA-damage in inner organs. On the other hand, the data show that MITC is not a harmless agent since it induces genotoxic effects in human derived cells at concentrations which are similar to those that lead to acute toxicity. Therefore, exposure of humans to concentrations which lead to acute toxic symptoms, e.g. by accidental release of MITC or its precursors into the environment, might lead to genotoxic effects in tissues that are directly exposed to the isothiocyanate*". The authors also suggest that the DNA-damaging activity of MITC could be, at least partly, related to lipid peroxidation/ oxidative stress.

According to the notifier, "*based on the known reaction of MITC with GSH, GSH depletion may be the main trigger in vitro to increase the cytotoxicity and following genotoxicity*". The depletion of GSH as a result of detoxification is well noted by the **RMS**, but is on itself an insufficient argument to ignore the *in-vitro* clastogenicity/ aneugenicity outcome. If the GSH-depletion were the only MoA, it could be anticipated that MITC would be even more genotoxic than metam, as the GSH-depletion is probably linked to the rapid binding of MITC with glutathione (as a phase II-detoxification step), and it would be expected that this mechanism would be even more prominent with MITC if applied directly, as compared to an exposure after being released by metam hydrolysis. A higher genoxicity of MITC seems not to happen, as the genotoxic burden seems to be more pronounced with metam than with MITC.

The other rationale of the notifier, to consider the positive genotoxicity results of both metam and MITC in the mammalian gene mutation assay irrelevant, on the basis of the hypothesis that CHO cell lines would display a deficiency in the p53 status, and thus of no relevance for the human was not considered justified from the scientific point of view by the RMS, in the light of positive findings in other p53-competent cells, at least for metam (not for MITC), indicating that at least the a.s. metam may be genotoxicologically active via another MoA.

However, in the case of MITC the positive genotoxicity findings were restricted to the *in-vitro* test systems. The positive finding in the CHO gene-mutation assay was not confirmed in bacterial cells (as regards the gene-mutation part), but was confirmed in the *in-vitro* micronucleus assay in the same cell line (as regards the clastogenicity/ aneugenicity part).

For completeness, it is worth noting that in one publication (Miyahara *et al*, 1997, see B.6.8.1.3.4), MITC was tested *in-vitro* (along with 15 other isothiocyanates) in a test battery to detect topoisomerase II inhibitors. Since some of the latter may be potent mutagens and carcinogens, it was appropriate to assess whether MITC could act through such a MoA. However, only 2/16 tested ICT's were TopII inhibitors at 12.5µg/mL, namely the allyl and *p*-tolylesters of isothiocyanic acid, but not MITC itself. Although the article is limited, but no other publication was found on this subject, the finding offers complementary information on the (absence of) potential genotoxicity of MITC.

In contrast, all ***in-vivo*** genotoxicity assays conducted with MITC were negative, including a comet assay where DNA damage was monitored in site-of-contact tissue (stomach) and in liver tissue. All *in-vivo* results are considered valid considering that adequate doses have been tested, and that MITC was demonstrated to be systemically available on a WoE basis.

Whereas it is proposed to discuss the need to classify **metam** as a **Muta 2** genotoxicant, it appears that the WoE points towards no classification for its main metabolite methylisothiocyanate (**MITC**).

In conclusion, RMS is of the opinion that MITC, in contrast to metam, was considered devoid of genotoxic potential *in-vivo*, and should thus not be classified accordingly.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Metam (incl. -sodium and -potassium):

Classified – MUTA , Category 2 (H341: "Suspected of causing genetic defects")

MITC:

No classification proposed

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity

Table 53a: Summary of long-term toxicity and carcinogenicity of Metam sodium

Type of test; test species, test substance – batch number – purity; tested doses Guidance, GLP,	Results			References
	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Findings	
2 year, drinking water LT toxicity /carcinogenicity study, rat (Hsd/Ola: Wistar-Tox) **Metam sodium** Batch n° BAS/005/OON 90-2 Purity: 525.54 g/L = 43.148% w/w Doses tested: 0, 0.019, 0.056, 0.19 mg/mL = 0, 1.5, 4.3,12.5 mg/kg bw/d (♂) 0, 2.7, 6.8, 16.8 mg/kg bw/d (♀) Equivalent to OECD 453 (1981) GLP compliant	Systemic: 1.5 Carcino: 1.5	Systemic: 4.3 Carcino: 4.3	↓body weight (♀), ↓food (♀) and ↓water consumption, haematological and urinalysis changes (♂,♀). ↑non-neoplastic lesions in the liver (spongiosis/peliosis) (♂,♀), adrenal vascular ectasy (♂), Harderian gland mononuclear cell infiltration (♂), Steno's gland atrophy (♂,♀) or adenitis (♀), rhinitis (♀), Bowman's gland /duct hypertrophy (♂), spleen porphyria (♂) and uterus glandular dilatation. *Top-dose:* ↑olfactory and respiratory epithelial hyperplasia (♂,♀), spleen haemosiderosis (♂,♀) and myopathy (♂). Considering animals surviving >1 year: trend-like ↑incidence of haemangioma + haemangiosarcoma (♂). *Top-dose* ↑ hepatocellular adenocarcinoma, and 1 hepatoblastoma (♂). Conclusion: **Carc 2, H351 «*Suspected of causing cancer*»**	[redacted] 1994 **B.6.5.1/01**
2 year, drinking water LT toxicity /carcinogenicity study, mouse (C57BL/10JfCD-1/Alpk) **Metam sodium** Batch n° BAS/005/00N 90-2 Purity: 525.54 g/L (43.148%) Doses tested: 0, 0.019, 0.074, 0.23 mg/mL bw/d. = 0, 1.9, 7.2 , 28.9 mg/kg bw/d (♂) 0, 2.6, 9.6 and 31.2 mg/kg bw/d (♀) Equivalent to OECD 451 (1981) GLP compliant	Systemic: <1.9 Carcino: <1.9	Systemic: 1.9 Carcino: 1.9	↑eosinophilic inclusion bodies in urinary bladder epithelial cells (♂,♀). *At 7.2 mg/kg bw/d and/or above:* ↓body weight (♂), food (♂) and water consumption (♂,♀), ↑liver w (♂,♀), ↑kidney weight (♀). ↑non-neoplastic urinary bladder lesions: ↑epithelial hyperplasia (♂,♀), ↑submucosal hyalinisation, ↑connective tissue at 7.2 mg/kg b.w./d (♀) and above (♂,♀). Liver toxicity: ↑vacuolation (♂,♀) at the top-dose and hepatocellular necrosis (♀) at 7.2 mg/kg b.w./d and above. *Top-dose:* ↑urinary bladder submucosal inflammatory cell infiltration (♂,♀). ↑angiosarcoma at any site (♂). *At 7.2 mg/kg bw/d and/or above:* ↑angiosarcoma, in the spleen (♂), in the liver (♀) and at any site (♂,♀). *Top-dose:* ↑transitional cell papilloma/carcinoma of the urinary bladder Conclusion: **Carc 2, H351 «*Suspected of causing cancer*»**	[redacted], 1994 **B.6.5.2/01**

Table 53b: Summary of long-term toxicity and carcinogenicity of MITC

Type of test; test species, test substance – batch number – purity; tested doses; Guidelines, GLP	Results			References Study n°
	NOAEL	LOAEL	Findings	
RAT				
2-year oral (drinking water) toxicity and carcinogenicity study, rat (Crl:CD (SD)). **MITC,** B.n°: 28 166; 29 482; Purity: 95.36% - 96.06% Doses tested: 0, 2, 10, 50 ppm 0, 0.08, 0.44, 1.60 mg/kg bw/d (♂) 0, 0.12, 0.66 and 2.65 mg/kg bw/d for (♀) OECD 453 (2018) Non GLP compliant	Systemic 0.44 mg/kg bw/d Carcinogenicity 1.60 mg/kg bw/d	Systemic 1.60 mg/kg bw/d Carcinogenicity >1.60 mg/kg bw/d	↓Body weight, altered WBC parameters, bone marrow hyperplasia, ↑kidney microcalculi, liver effects, and spleen hyperplasia/ ↑haematopoiesis No treatment-related ↑tumour incidence observed	[redacted], 1984 **B.6.8.1.4.1/ 01**
2-year inhalation chronic toxicity and carcinogenicity study, rat (Crl:CD(SD)) **MITC,** B. n°: 56198PJV; Purity: 97.2% - 99.7% Doses tested: 0, 0.5, 5, 20 ppm 0, 1.5, 15, 60 mg/m³ 0, 0.65, 6.5, 26 mg/kg b.w./d OECD 453 (2018) GLP compliant	Local <0.5 ppm <1.5 mg/m³ <0.65 mg/kg bw/d Systemic 5 ppm 15 mg/m³ 6.5 mg/kg bw/d Carcinogenicity 5 ppm 15 mg/m³ 6.5 mg/kg bw/d	Local 5 ppm 1.5 mg/m³ 0.65 mg/kg bw/d Systemic 20 ppm 60 mg/m³ 26 mg/kg bw/d Carcinogenicity 20 ppm 60 mg/m³ 26 mg/kg bw/d	↑squamous metaplasia , ↑olfactory nasal epithelium degeneration, ↑epithelial hyperplasia, ↑squamous metaplasia on the larynx. *At higher doses,* ↑eyes opacity,bilateral keratitis and various adverse findings in nasal tissues, larynx, trachea, lungs, olfactory bulbs. No ↑mortality *vs.* control incidence, but at top-dose death caused by nasal tumours. Other relevant effects: ↓body weight (>20%), ↓body weight gain, ↑haematology/clinical chemistry findings. ↑malignant and benign nasal tumours, 1 benign papilloma in the lung Possible MoA: corrosive action of MITC, no *in-vivo* genotoxicant, but human relevance not excluded. Conclusion: carcinogenic in the rat after administration of MITC via inhalation. **Carc 2, H351 «*Suspected of causing cancer*»**	[redacted], 2015a **B.6.8.1.4.1/ 02**

Type of test; test species, test substance – batch number – purity; tested doses; Guidelines, GLP	Results			References Study n°
	NOAEL	LOAEL	Findings	
MOUSE				
2-year chronic oral (drinking water) toxicity and carcinogenicity study, Mouse (ICR:JCL) **MITC**, B. n° MS 25206; Purity: 93.14% Doses tested: 0, 5, 20, 80, 200 ppm 0, 0.82, 3.3, 11.83, 25.71 mg/kg bw/d (♂) 0, 0.91, 3.66, 13.03, 29.03 mg/kg bw/d (♀) OECD 453 (2018) Non GLP compliant	Systemic 20 ppm 3.3 mg/kg bw/d Carcinogenicity 200 ppm 25 mg/kg bw/d	Systemic 80 ppm 12 mg/kg bw/d Carcinogenicity >200 ppm >25 mg/kg bw/d	↑Clinical signs, ↓body weight, ↓body weight gain, slight effects in blood, and altered organ weights. No treatment-related ↑tumour incidence observed Not carcinogenic in the mouse after oral administration of MITC.	[redacted] 1980 **B.6.8.1.4.2/ 01**
18-month inhalation carcinogenicity study, Mouse (Crl:CD-1) **MITC,** B. n°: 56198PJV; Purity: 97.2% - 99.7% Doses tested: 0, 1, 5, 15 ppm 0, 3, 15, 45 mg/m³ 0, 1.35, 6.7, 20 mg/kg b.w./d OECD 453 (2018) GLP compliant	Local <1 ppm <3 mg/m³ <1.35 mg/kg bw/d Systemic 1 ppm 3 mg/m³ 1.35 mg/kg bw/d Carcinogenicity 15 ppm 45 mg/m³ 20 mg/kg bw/d	Local 1 ppm 3 mg/m³ 1.35 mg/kg bw/d Systemic 5 ppm 15 mg/m³ 6.7 mg/kg bw/d Carcinogenicity >15 ppm 45 mg/m³ 20 mg/kg bw/d	↑respiratory and transitional hyperplasia of nasal epithelium; Dose-dependent ↑nasal findings (incidence and severity, minimal to mild) ↓body weight, ↓body weight gain, ↓spleen weights (♂) Neoplastic finding Single incidence of (benign) papilloma, insufficient for classification. Not carcinogenic in the mouse after administration of MITC via inhalation.	[redacted], 2015b **B.6.8.1.4.2/ 02**

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Epidemiological study (open scientific literature)	**Metam-sodium**	Case-control study including 2189 case children and 4335 controls matched for birth date and sex. Authors estimated the *in utero* exposure potential from specific chemicals and chemical groups (invluding a.o. metam-sodium) used in the 9 months before birth within *ca.* 800m of the maternal residence. Odds ratios (ORs) using conditional logistic regression were calculated.	An approximate 2-fold increase in childhood cancer incidence, however, the authors themselves were very prudent in suggesting any causality, as the few elevated risk associations in this study were possibly consistent with chance, given the large number of comparisons. It is further notable that since then, no meaningful epidemiological study explicitly pointed to the association between metam-sodium use as fumigant and cancer incidence. Their conclusion that *«the evidence of considerable MITC drift suggests that the association between metam sodium use and leukemia risk deserves more careful consideration in future studies»* is useful for further projects regarding product stewardship and (bio) monitoring studies in future.	**Reynolds *et al,* 2005** (see also summary in Vol.3, B.6, B.6.9.4/10)

Epidemiological study (open scientific literature)	**Metam-sodium**	Evaluation the potential geospatial relationship between agricultural pesticide use and two cancer metrics (pediatric cancer incidence and total cancer incidence) across 11 contiguous states in the Western US. Pesticide usage data: collected from the U.S. Geological Survey Pesticide National Synthesis Project database, Cancer data: National Cancer Institute State Cancer Profiles.	At the state level, this study identified significant relationships between total mass of fumigants applied and pediatric cancer incidence and between the mass of metam and total cancer incidence. At the county scale, significant associations between total fumigant mass, high and medium tertiles of fumigant mass, total pesticide mass, and high tertiles of pesticide mass relative to total cancer incidence was identified. Further, a multilevel model was developed using fumigant mass and fumigant mass tertiles across both states and counties, which predicted total cancer incidence at the county scale. The fumigant application rate was shown to be important relative to total cancer and pediatric cancer incidence; moreover, this relationship was maintained regardless of whether the spatial resolution used in the analysis was conducted at the state or county level. The authors however recognised limitations of this study, including: -the lack of temporal continuity between the secondary data sets used to conduct the analysis presented (2017 pesticide data vs. 2012–2016 5-year average cancer incidence data). -other variables (like any demographic factors, such as employment in the agricultural sector or race and ethnicity that could potentially explain the observed relationships between pesticide use and rates of all-cancer and pediatric cancer incidences) were not incorporated. -individual-level exposure were not measured, thus it only constitutes a basis for future scope to overcome these limitation and collect additional temporal data sets on metam and cancer metrics to perform exposure-based assessments. For the RMS, the study is restricted to rather crude comparisons between potential fumigant/metam use and cancer incidence, extracted from publicly available databases. With these limitations in mind, the results of this study should be interpreted with caution. The authors noted that while the outcomes surrounding fumigant use are suggestive, more information is needed to determine whether the use of the fumigant metam is directly linked to cancer.	**Joseph *et al*, 2022** (see also summary in Vol.3, B.6, B.6.9.4/11)
MITC: no data available				

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

METAM sodium

All studies provided in this section have already been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. As since the Annex I inclusion for metam the data requirements have been adapted and further studies were performed with metam and MITC a re-evaluation of all studies was performed. Furthermore, information on historical control data was provided.

(i) Metam sodium was administered to **rats** in drinking water for 2 years ([redacted], 1994, **B.6.5.1/01**).

In this 2-yr rat carcinogenicity study, most adverse general toxicity findings were observed at the two highest doses of 4.3 mg/kg b.w./d and 12.5 mg/kg b.w./d, including decreases of body weight (♀), food (♀) and water consumption, haematological and urinalysis changes (♂,♀). The severity of all these changes was such that the top-dose would be qualified as sufficient in terms of carcinogenicity assessment, but certainly not excessive.

Dose-related increases in non-neoplastic lesions in the liver (spongiosis/peliosis) (♂,♀), adrenal vascular ectasy (♂), Harderian gland mononuclear cell infiltration (♂), Steno's gland atrophy (♂,♀) or adenitis (♀), rhinitis (♀), Bowman's gland /duct hypertrophy (♂), spleen porphyria (♂) and uterus glandular dilatation were observed at 4.3 mg/kg b.w./d and above.

Top-dose findings included increased olfactory and respiratory epithelial hyperplasia (♂,♀), spleen haemosiderosis (♂,♀) and myopathy (♂).

Considering animals surviving beyond 1 year of exposure, and summing up the incidences of haemangioma and haemangiosarcoma, a trend towards increasing incidence of these tumours was observed in the ♂ at 4.3 mg/kg b.w./d and above.

Top-dose ♂ suffered an increased incidence of hepatocellular adenocarcinoma, and one hepatoblastoma was observed.

Table 55a: Incidence of selected tumours in rats administered metam sodium ([redacted], 1994).

		♂				♀			
Dose[mg/mL]		**0**	**0.019**	**0.056**	**0.19**	**0**	**0.019**	**0.056**	**0.19**
Dose [mg/kg bw/d]		**0**	**1.5**	**4.3**	**12.5**	**0**	**2.7**	**6.8**	**16.8**
	N	*64*	*64*	*64*	*64*	*64*	*64*	*64*	*64*
Liver hepatocellular adenocarcinoma	#	3	2	4	**5**	0	0	2	0
	%	*4.7*	*3.1*	*6.3*	***7.8***	*0.0*	*0.0*	*3.1*	*0.0*
Liver hepatoblastoma	#	0	0	0	**1**	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	*1.6*	*0.0*	*0.0*	*0.0*	*0.0*
Mesenteric lymph node **haemangiosarcoma**	#	0	2	**5**	2	0	3	1	0
	%	*0.0*	*3.1*	***7.8***	*3.1*	*0.0*	*4.7*	*1.6*	*0.0*
		HCD: 0-4 %				**HCD: 0-3.9 %**			
Mesenteric lymph node **haemangioma**	#	9	3	3	8	2	1	3	0
	%	*14.1*	*4.7*	*4.7*	*12.5*	*3.1*	*1.6*	*4.7*	*0.0*

For these reasons, both the systemic rat **long-term toxicity NOAEL** and the **carcinogenicity NOAEL** is set at **1.5 mg/kg b.w./d**, disregarding less concerning findings at this dose, including sporadic decreases in water consumption, haematological/clinical chemistry findings of uncertain toxicological relevance, and a slight increase of uterus glandular dilatation incidence at the lowest dose.

The presence of the liver adenocarcinoma/hepatoblastoma and the increased incidence of haemangioma /haemangiosarcoma in the rat may trigger the proposal to classify the a.s. metam sodium as **Carc 2**, H351 «*Suspected of causing cancer*».

RMS acknowledges that the latter tumour incidence shows limited dose-response, but the presence of similar tumours in the mouse, also considering the potential MoA via oxidative stress, is considered sufficient not to disregard completely this vascular tumour finding in the rat.

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (rat) = 1.5 mg/kg bw/d (metam sodium).

(ii) Metam sodium was also administered to **mice** in drinking water for 2 years ([redacted], 1994, **B.6.5.2/01**).

In this 2-yr mouse carcinogenicity study, most adverse general toxicity findings were observed at the two highest doses of 7.2 mg/kg b.w./d and 28.9 mg/kg b.w./d, including occasional decreases of body weight (♂), food (♂) and water consumption (♂,♀), increased liver (♂,♀) and kidney weight (♀).

Dose-related increases in non-neoplastic urinary bladder lesions, including epithelial hyperplasia (♂,♀) and submucosal hyalinisation/ increased connective tissue observed at 7.2 mg/kg b.w./d (♀) and above (♂,♀). Top-dose findings included submucosal inflammatory cell infiltration (♂,♀).
While pathological significant changes in the urinary bladder were mainly present at the two highest doses, eosinophilic inclusion bodies were present in increased amounts in the epithelial cells of all treated groups (including the lowest tested dose of 1.9 mg/kg b.w./d).
Liver toxicity was evident through an increased incidence of fatty vacuolation (♂,♀) at the top-dose and areas of hepatocellular necrosis (♀) at 7.2 mg/kg b.w./d and above.

Transitional cell papilloma/carcinoma of the urinary bladder affected only two mice at the top dose, but taking into account the underlying non-neoplastic findings in this organ, it should be discussed whether these single lesions should be regarded substance-related.
At the two highest doses, there was an increased incidence of angiosarcoma, especially in the spleen (♂) and in the liver (♀). The overall incidence of mice with angiosarcoma in any site was dose-dependently increased at the dose of 1.9 mg/kg b.w./d (♂) and above (♂,♀).

Table 55b: Incidence of selected tumours in mice administered metam sodium ([redacted], 1994).

	♂				♀			
Dose [mg/mL]	**0**	**0.019**	**0.074**	**0.23**	**0**	**0.019**	**0.074**	**0.23**
Dose [mg/kg bw/d]	**0**	**1.9**	**7.2**	**28.9**	**0**	**2.6**	**9.6**	**31.2**
Angiosarcoma								
Spleen angiosarcoma	5/55	2/55	8/55	15/55	0/55	1/55	3/55	4/55
% affected	*9.1*	*3.6*	***15.5▲***	***27.3▲***	*0*	*1.8*	***5.5***	***7.3***
	HCD: 0 - 9.1 %				**HCD: 0 - 9.1 %**			
Liver angiosarcoma	0/55	6/55	3/55	7/55	0/55	0/55	1/55	3/55
% affected	*0*	*10.9*	*5.4*	*12.7*	*0*	*0*	***1.8***	***5.4***
	HCD : 0-12%				**HCD : 0-8%**			
Subcutaneous tissue angiosarcoma	1/55	1/55	1/55	3/55	0/55	1/55	1/55	3/55
% affected	*1.8*	*1.8*	*1.8*	*5.5*	*0*	*1.8*	*1.8*	*5.5*
	HCD: 5.9 (summarised incidence over all studies)				**HCD: 8.1** (summarised incidence over all studies)			
Bone marrow angiosarcoma	0/55	2/54	0/54	0/55	0/55	0/55	0/54	0/54
% affected	*0.0*	*3.6*	*0*	*0*	*0*	*0*	*0*	*0*
	HCD: 0				**HCD: 0 – 2.1**			
LN mesenteric angiosarcoma	0/54	1/55	0/55	0/54	0/54	0/55	0/50	0/55
Mesentery angiosarcoma	0/0	0/0	0/0	**1/1**	0/2	0/1	0/1	0/1
Thoracic angiosarcoma	1/1	0/0	0/0	0/0	0/0	0/	0/1	0
Ovary angiosarcoma	-	-	-	-	2/55	0/55	0/52	0/55
Uterus angiosarcoma	-	-	-	-	0/55	0/55	1/54	0/55
Limb angiosarcoma	0/1	0/1	0/2	**1/4**	1/2	0/1	0/2	0/2
Sternum angiosarcoma	0/54	0/53	0/55	0/55	1/55	0/55	0/54	0/55
Overall incidence of mice with angiosarcoma in any site								
number of animals affected	7/55	**12/55**	**12/55**	**27/55****	4/55	2/55	**6/55**	**10/55**
%affected	*12.7*	***21.8***	***21.8***	***49.1***	*7.3*	*3.6*	***10.9***	***18.2***
Urinary bladder								
transitional cell carcinoma **M**	0/54	0/55	0/55	0/55	0/54	0/52	0/53	**1/55**
transitional cell papilloma **B**	0/54	0/55	0/55	**1/55**	0/54	0/52	0/53	0/55

Total incidences (intercurrent + terminal); Absolute alues expressed as number affected tissue/number of tissues examined;
* $p \leq 0.05$; ** $p \leq 0.01$: **B**: benign - **M**: malignant ; LN : lymph node; **▲**: above HCD range; **B** : benign - **M**: malignant

Therefore, the mouse **long-term toxicity LOAEL** was reconsidered by the RMS and established at 1.9 mg/kg b.w./d, taking into account the dose-related increased of eosinophilic cytoplasmic inclusion in the urinary bladder epithelium incidence at *all* tested doses.
It was also considered that the **carcinogenicity LOAEL** should also be revised downwards, on the basis of the overall incidence of mice with angiosarcoma at any site, although is it acknowledged that organ-specific angiosarcoma (liver, spleen) was only detected at 7.2 mg/kg b.w./d onwards. Considering the general character of the lesion, the latter takes precedence on the concern of any site-specific angiosarcoma appearance, and the conclusion on the LOAEL for mouse carcinogenicity, seems justified.

The presence of the urinary bladder papilloma/carcinoma and the increased incidence of angiosarcoma in the mouse triggers the proposal to classify the a.s. metam sodium as **Carc 2**, H351 «*Suspected of causing cancer*».

EU-agreed endpoint (EFSA Journal 2011;9(9):2334): NOAEL (mice) = 1.9 mg/kg bw/d (metam sodium).

In both the Taminco and the Lainco dossier, it is mentioned: Metam sodium does not have a harmonised classification for carcinogenicity. However, it is proposed in the CLH dossier submitted by Taminco bvba that, based on the available data, metam should be classified in Carcinogenicity Category 2 (H351: Suspected of causing cancer).

MITC

Chronic drinking water studies in rat ([redacted], 1984, **B.6.8.1.4.1/01**) and mouse ([redacted] 1980, **B.6.8.1.4.2/01**) have already been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. As since the Annex I inclusion for metam the data requirements have been adapted a re-evaluation of the studies was performed. Furthermore, information on historical control data was provided.

Additional chronic studies were performed via the inhalation route in rats ([redacted] 2015a, **B.6.8.1.4.1/02**) and mice ([redacted], 2015b, **B.6.8.1.4.2/02**).

(i) RAT

In [redacted] (1984, **B.6.8.1.4.1/01**), rats received MITC via drinking water at 2, 10 or 50 ppm over 104 weeks. Reduced body weight of ♂ rats, was more important at top dose at the end of the study period, white blood cell parameters, histopathological findings such as bone marrow hyperplasia, increased kidney microcalculi, liver effects, and spleen hyperplasia/ increased haematopoiesis were reported at 50 ppm (1.6 mg/kg bw/d) and could be related to MITC exposure. A **systemic NOAEL= 10 ppm (0.44 mg/kg bw/d)** is proposed.

MITC is not carcinogenic under these experimental conditions, and a carcinogenicity NOAEL = **50 ppm (1.60 mg/kg bw/d)** is set accordingly.

In [redacted] (2015a, **B.6.8.1.4.1/02**), where MITC was administered to SD rats by inhalation during 2 years at doses of 0, 0.5, 5 and 20 ppm, no statistically significant effect on the survival was noted for neither ♂ nor ♀ rats in any group at termination. However, the top-dose animals beared squamous cell carcinoma and anaplastic carcinoma of the nose, as well as lung necrotising or suppurative inflammatory lesions, secondary to MITC-related injury. While survival rate was equally distributed among the treated animals, the cause of death in these top-dose group was mainly the presence of nasal tumours.

Thin appearance, associated with lower body weight and food consumption, also had a higher occurrence and incidence in the 20 ppm ♂ and ♀. In the 5 ppm group and above, rales and laboured respiration were observed with a slightly higher occurrence and/or incidence in ♂ and ♀. MITC exposure-related palpable masses were located on or near the nose in top-dose ♂ and ♀.

Based on mean body weights and cumulative body weight gains, the high-concentration of 20 ppm corresponded to or exceeded the maximum tolerated dose (MTD).

Treatment-related clinical and anatomic pathology findings noted prior to or at the time of the chronic toxicity evaluation (study week 52) included changes in haematology and clinical chemistry parameters, organ weight effects (higher lung weights, secondary higher adrenal gland weights, and secondary stress-related lower spleen and thymus weights) and microscopic lesions in the nasal cavity, larynx, trachea, and lung. These lesions resulted from direct contact injury to mucosal epithelium by MITC, had a higher incidence and were most severe at the top-dose.

The systemic **NOAEL** (*i.e.* effects mostly outside the respiratory tract) is **5 ppm,** on the basis of decreased body weight (gain), haematology and clinical chemistry parameter modifications, and organ weight effects.

Non-neoplastic histopathological findings along the upper airway tract at 0.5 ppm and above were squamous and respiratory epithelial metaplasia, and olfactory epithelial degeneration in nasal tissues, and epithelial hyperplasia and squamous metaplasia in the larynx. Other non-neoplastic nasal, laryngeal, tracheal and lung lesions were considered adverse in the 5 and/or 20 ppm group.

Other treatment-related microscopic findings outside of the respiratory tract were in the olfactory bulb (brain) and eyes. Olfactory bulb effects were a direct consequence of nasal olfactory epithelial degeneration and atrophy, while adverse effects in the eyes were a consequence of direct contact with the irritant MITC.

The **local NOAEL** is **< 0.5ppm,** based upon increased incidences of squamous metaplasia and olfactory epithelial degeneration in the nasal epithelium, and epithelial hyperplasia and squamous metaplasia on the larynx. It seems evident that the observed nasal effects at the lowest dose are part of a gradient which incidence and severity grade increased at the next-higher doses. Toxicologically significant local effects (portal of entry) were observed clinically

in the eyes (opacity and bilateral keratitis in 20 ppm group) and microscopically in the nasal tissues, larynx, trachea, lungs, olfactory bulbs, and eyes.

MITC exposure-related *neoplastic findings* were found in the 20 ppm group ♂ and ♀ and included malignant and benign nasal tumours and a single benign papilloma in the lung (1♂).

Table 55c: Incidence of nasal tumours (all levels combined)* in rats administered MITC by inhalation ([redacted], 2015a).

		♂				♀			
Dose [ppm]		**0**	**0.5**	**5**	**20**	**0**	**0.5**	**5**	**20**
Number of animals examined		*60*	*60*	*60*	*60*	*60*	*60*	*60*	*60*
M Carcinoma, anaplastic	#	0	0	0	**2**	0	0	0	**2**
	%	*0.0*	*0.0*	*0.0*	***3.3***	*0.0*	*0.0*	*0.0*	***3.3***
M Carcinoma, squamous cell	#	0	0	0	**15**	0	0	0	**15**
	%	*0.0*	*0.0*	*0.0*	***25.0***	*0.0*	*0.0*	*0.0*	***25.0***
M Carcinoma, squamous cell, multiple	#	0	0	0	1	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*	*0.0*
M Carcinosarcoma	#	0	0	0	1	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*	*0.0*
M Schwannoma, malignant	#	0	0	0	0	0	0	0	1
	%	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*1.7*
B Adenoma	#	0	0	0	**3**	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	***5.0***	*0.0*	*0.0*	*0.0*	*0.0*
B Papilloma	#	0	0	0	**6**	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	***10.0***	*0.0*	*0.0*	*0.0*	*0.0*
B Papilloma, squamous	#	0	0	0	1	0	0	0	0
	%	*0.0*	*0.0*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*	*0.0*

* excluding extension of nasal tumours into surrounding tissue and metastatic tumours; # = number of animals with neoplasm; % = number of animals with neoplasm related to total number of animals; B = benign; M = malignant; S = metastatic

Table 55d: Incidence of non-neplastic nasal findings (all levels combined) in rats administered MITC by inhalation ([redacted], 2015a).

	♂				♀			
Dose [ppm]	**0**	**0.5**	**5**	**20**	**0**	**0.5**	**5**	**20**
Number of tissues examined	*60*	*60*	*60*	*60*	*60*	*60*	*60*	*60*
Atrophy, Bowman's glands	0	0	28	57	0	1	23	56
Atrophy, olfactory epithelium	0	0	0	53	0	1	2	49
Atrophy, olfactory nerve bundle	0	0	32	58	0	1	34	56
Collapse, dorsal meatus	0	0	0	5	0	0	0	3
Degeneration, olfactory epithelium	4	5	47	33	1	**5**	**43**	**46**
Degeneration, respiratory epithelium	1	0	1	1	0	1	0	0
Degeneration, transitional epithelium	0	0	0	0	0	1	0	0
Dilatation, Bowman's glands	0	0	0	13	0	0	0	20
Dilatation, lumen	1	**2**	**4**	**21**	0	0	0	**13**
Exudate, inflammatory	21	20	33	60	11	10	21	60
Hyperplasia, Bowman's glands	0	0	0	2	0	0	0	0
Hyperplasia, olfactory epithelium	0	1	0	0	0	0	0	0
Hyperplasia, respiratory epithelium /mucous cell	39	34	**50**	**60**	24	26	**44**	**59**
Hyperplasia, squamous epithelium	8	3	**10**	**54**	11	3	**16**	**55**
Hyperplasia, transitional epithelium	47	45	59	44	23	**40**	**52**	**47**
Inflammation, chronic active	44	31	53	59	29	30	51	59
Metaplasia, respiratory epithelium	0	**1**	**3**	**60**	0	**1**	**4**	**58**
Metaplasia, squamous	8	**20**	**38**	**60**	3	2	**19**	**60**
Mineralisation	0	0	1	9	0	0	1	10
Necrosis, squamous epithelium	0	0	0	**5**	0	0	**2**	**3**

Results expressed as number of animals with findings, all animals combined

The **carcinogenicity NOAEL is 5 ppm,** based upon increased incidences of squamous cell carcinoma (25%) and anaplastic carcinoma (about 3%) in both ♂ and ♀, adenoma (5%) and papilloma (10%) in the ♂ **at the top-dose of 20 ppm**. Invasive extensions of the carcinoma were found at the level of neighbouring tissues, as well as metastases in the mandibular lymph node. In addition, a single lung papilloma (♂) is observed at this dose as well.

It should be discussed if the tumours, obviously triggered by the corrosive action of MITC in the nasal linings, would justify a classification **Carc Cat 2**, H351 **«*Suspected of causing cancer*».** It is acknowledged that MITC is only found to be carcinogenic at the highest dose(s) used in the lifetime rat bioassay, and the characteristics associated with doses exceeding the MTD as outlined above are present, this could be an indication of a confounding effect of excessive toxicity. This may support either a classification of MITC in Category 2 or no classification. Under CLP, when such a mechanism is identified then classification may not be appropriate. Only if a mode of action of tumour development is conclusively determined *not* to be operative in humans may the carcinogenic evidence be discounted. It may be discussed whether such MoA of tumour formation is relevant to humans or not, but in **RMS** opinion, a primary irritation of the upper airways is not *a priori* to be considered intrinsically irrelevant for the human, and several caustic substances are known which cause upper respiratory airway irritation without being classified for cancer. It is further notable that the tumour formation of MITC starts at about 1 year exposure, increasing in incidence at severity with time, and responsible for the death of animals bearing it from that time-point and afterwards.

The notifier further considered that, based on low incidence and/or severity (generally minimal to mild), presence in only 1-2 nasal levels or 1 laryngeal level and/or multifocal appearance, non-neoplastic MITC-related findings were not considered adverse in the lowest dose group of 0.5 ppm. In notifier's opinion, MITC-related non-neoplastic nasal lesions were considered adverse in the 5 and 20 ppm group ♂ and ♀ (for the 5 ppm group, based on incidence and severity of squamous metaplasia and olfactory epithelial degeneration). Laryngeal findings were considered adverse in the 5 ppm ♂ (squamous metaplasia and epithelial hyperplasia) and 20 ppm group ♂ and ♀. MITC-related non-neoplastic findings in the lungs, trachea, eyes, and olfactory bulbs were considered adverse in the 20 ppm group.

Regarding the neoplastic findings, notifier stated
« For the active substance renewal of metam more recent carcinogenicity studies via the inhalation route in rats and mice are available for MITC. A treatment-related increase in tumours in the nasal cavity and associated tissues of ♂ and ♀ rats was apparent in a 2-year carcinogenicity study via inhalation exposure. However, these findings were observed at doses exceeding the MTD and only in tissues with direct substance exposure. Therefore, these findings were considered secondary to the known cytotoxic/corrosive properties of MITC. No statistical significant neoplastic findings were noted in ♂ and ♀ mice of an 18-month inhalation carcinogenicity study. Based on the available information no classification for carcinogenicity according to CLP is warranted for MITC. Furthermore, the potential corrosive (cytotoxic) effects of MITC are already considered by a classification proposal for MITC for acute toxicity, skin corrosion (Cat. 1) and STOT RE. In conclusion, no intrinsic hazard for carcinogenicity of MITC is expected and therefore no classification for carcinogenicity is proposed.»

(ii) MOUSE

In [redacted] (1980, **B.6.8.1.4.2/01**), mice received MITC in drinking water at 5, 20, 80 and 200 ppm.
A NOAEL = 20 ppm (3.3 mg/kg bw/d) is proposed, taking into account the different slight effects seen at 80 ppm (12 mg/kg bw/d) such as the increased incidence of clinical signs, slight decreased body weight and body weight gain, slight effects in blood and altered organ weights. Based on this study, MITC is not carcinogenic after administration in the drinking water in mice.
A **carcinogenicity NOAEL = 200 ppm (25 mg/kg bw/d)** may be proposed.

In [redacted] **(2015b, B.6.8.1.4.2/02)**, CD-1 mice received 0, 1, 5 and 15 ppm (corresponding to 0, 3, 15 and 45 mg/m³) by whole-body inhalation. Although the 15 ppm high concentration exceeded the MTD (based on low mean body weight and cumulative body weight gain), there were no MITC exposure-related early deaths and no negative effects on survival.
The systemic **NOAEL** (*i.e.* effects outside the respiratory tract) = **1 ppm**, based on lower mean body weight and body weight gain, lower food consumption and decreased spleen weight at **5 ppm (=15 mg/m³)** and above.
Toxicologically significant local effects were observed clinically in the eyes (opacity in 15 ppm) and microscopically in the nasal tissues, olfactory bulbs, larynx, trachea and the cornea of the eyes.
In the nasal epithelium, the incidence of both respiratory and transitional epithelial hyperplasia was meaningfully and dose-dependently increased from the lowest dose of 1 ppm onwards, in both ♂and ♀.
Therefore, the **local NOAEL <1 ppm (<3 mg/m³)**, on this basis. However, it is also clear that most other histopathological findings were elevated at 5ppm and 15 ppm.

There was a single nasal neoplasm that consisted of a benign, exophytic papilloma in nasal level 1 in a 15 ppm group ♀ at the 18-month terminal necropsy. The papilloma was located on the nasoturbinate in an area of treatment-related

respiratory epithelial metaplasia. Although very rare (Brown *et al.*, 1991), spontaneous nasal neoplasms have been reported in mice. At top-dose, the incidence of Harderian gland hyperplasia (♂) was increased, but adenoma/carcinoma incidence was not elevated.
It was considered that there was insufficient evidence to indicate that MITC is carcinogenic in the CD-1 mouse. Therefore the **carcinogenicity NOAEL = 15 ppm** (=45 mg/m³).

Cited reference:

Brown HR, Monticello TM, Maronpot RR, Randall HW, Hotchkiss JR, Morgan KT. Proliferative and Neoplastic Lesions in the Rodent Nasal Cavity, Toxicol Pathol. 1991;19(4,1):358-72, 1991.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Metam sodium
As discussed hereabove (2.6.5.1), the presence of the liver adenocarcinoma/hepatoblastoma and the increased incidence of haemangioma /haemangiosarcoma in the rat may trigger the proposal to classify the a.s. metam sodium as **Carc 2, H351 «Suspected of causing cancer»**. The presence of the urinary bladder papilloma/carcinoma and the increased incidence of angiosarcoma in the mouse triggers the proposal to classify the a.s. metam sodium as **Carc 2, H351 «Suspected of causing cancer».**

Following criteria were checked for metam-sodium carcinogenicity studies in experimental animals
Carc. Cat 1B
«– sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or
(b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites»

The increased incidence of haemangiosarcoma in the drinking water study with metam-sodium in the **mouse** seems evident, although a significant increase is only evident in the spleen. However, taken together, the presence of a dose-dependent increase of haemangioma/haemangiosarcoma at any site further indicates a treatment-related induction of the tumours. The single incidence of urinary bladder carcinoma (♀) and papilloma (♂) is of uncertain toxicological relevance, although it should be discussed in the light of the presence of meaningfully elevated urinary bladder epithelial hyperplasia in both the ♂ and the ♀.
The increased incidence of haemangiosarcoma in the drinking water study with metam-sodium in the ♂ **rat** is only significant at the mid-dose. Considering the dose-response in the group of surviving animals (18,12,24,22% at resp. 0, 1.5, 4.3 and 12.5 mg/kg bw/d), the actual involvement of the treatment is uncertain.
However, taking into account the presence of haemangiosarcomas in the mouse, the carcinogenic potential of metam-sodium cannot be completely excluded either.
In the liver of the ♂ rats, a slight trend of elevated hepatocellular adenocarcinoma (5,3,6,8% at resp. 0, 1.5, 4.3 and 12.5 mg/kg bw/d), and one top-dose hepatoblastoma was noted. The incidences lacked statistical significance and the dose-response was rather weak.
Overall, RMS would not consider metam-sodium eligible for classification as Carc Cat 1B.

Carc. Cat.2
«– limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g.
(a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.»
RMS considers that there is evidence for a clear carcinogenic effect in the **mouse**, while the outcome in the **rat** is less convincing.

Further considerations are mentioned in Annex I: 3.6.2.2.6 of the CLP guidance, which may be taken into consideration, when assessing the overall level of concern are:

(a) tumour type and background incidence:

Essentially angiosarcoma in the mouse at many sites but clearly dose-dependent in the spleen of both ♂ and ♀.

(b) multi-site responses;

Except for the abovementioned angioarcoma, no evidence of multi site response in neither rat nor mouse. The single incidence of urinary bladder tumours in the mouse remains to be discussed, as it could be related to induced hyperplasia.

(c) progression of lesions to malignancy;

Yes, angiosarcoma are considered malignant

(d) reduced tumour latency;

No evidence of reduced latency

(e) whether responses are in single or both sexes;

Both sexes for angiosarcoma, although more pronounced in the ♂

(f) whether responses are in a single species or several species;

clear carcinogenic effect in the mouse, while the outcome in the rat is less convincing.

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

None known; it is of note that the pattern of tumours found in metam-sodium is not replicated in the studies conducted with its main metabolite MITC via the same route of administration (see below)

(h) routes of exposure;

Oral administration: effects seen in drinking water studies

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

No evidence of differences between rodents and human

(j) the possibility of a confounding effect of excessive toxicity at test doses;

The systemic toxicity and carcinogenicity NOAEL are established at the same dose. While the angiosarcoma incidence in the spleen tissue of the mouse is exacerbated at top-dose (about 29 mg/kg bw/d), the tested dose is not excessively toxic, and there is no particular indication of toxicity confounding the interpretation of the vessel tumours.

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

The MoA for the observed elevation of angiosarcoma incidence remains uncertain. Metam-sodium is in RMS opinion, unlikely to be classified as a mutagen, although the a.s. may display some genotoxicity at high doses, possibly related to the induction of oxidative stress. The implication of GSH depletion is also not excluded. On the other hand, while the main metabolite MITC is also inducing GSH depletion, it is noted that this metabolite does not induce the same type of vessel tumours as the parent compound metam-sodium, suggesting that some other MoA may be involved.

It is noted that only 2 studies on human populations (one case-control study, and one cross-sectional epidemiological study) were identified in the open public literature. The latter included a geospatial relationship between agricultural pesticide use and two cancer metrics (pediatric cancer incidence and total cancer incidence), mentioning a.o. metam sodium.

One study (Reynolds *et al*, 2005) suggested an elevated risk (OR=2.05 (95%CI: 1.01-4.17) for leukemia.

In a more recent second study (Joseph *et al*, 2022) there was a crude statistical association between the total quantity of metam applied and the general cancer incidence.

To the RMS, grossly in line with authors opinion, the results of these epidemiological studies should be interpreted with caution. The authors noted that while the outcomes surrounding fumigant use are suggestive, more information is needed to determine whether the use of the fumigant metam (and especially the actual quantity to which operators and residents would be exposed) is causally linked to cancer. In neither study, it is possible to conclude on any causal relationship between exposure to metam and cancer. They are therefore considered of limited value for the classification of metam-sodium or metam-potassium, notwithstanding the conclusions regarding the possible classification in animal studies.

MITC
As discussed hereabove (2.6.5.1), it could be discussed if the tumours, obviously triggered by the corrosive action of MITC in the nasal linings, would justify a classification **Carc Cat 2, H351 «Suspected of causing cancer».** On the other hand, it was considered that there was insufficient evidence to indicate that MITC is carcinogenic in the CD-1 mouse.

Following criteria were checked for MITC carcinogenicity studies in experimental animals
Carc. Cat 1B
«– sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or
(b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites»

There was no treatment-related increase of neither benign nor malignant tumours in the drinking water study with MITC in the **rat** and the **mouse**.
In contrast, MITC administered by inhalation proved carcinogenic for the nasal epithelium at the top-concentration in the **rat**, and could be defined as a site-of-contact MoA, associated with the known corrosive characteristic of MITC to the upper respiratory tract (URT).

Overall, RMS would not consider MITC eligible for classification as Carc Cat 1B.

Carc. Cat.2
«– limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g.
(a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.»
RMS considers that there is evidence for a clear carcinogenic effect in the **rat**, but not in the **mouse.**

Further considerations are mentioned in Annex I: 3.6.2.2.6 of the CLP guidance, which may be taken into consideration, when assessing the overall level of concern are:
(a) tumour type and background incidence:
Squamous cell and anaplastic carcinoma of the nasal respiratory epithelium in the rat in both ♂ and ♀.
(b) multi-site responses;
No
(c) progression of lesions to malignancy;
Yes
(d) reduced tumour latency;
No evidence of reduced latency
(e) whether responses are in single or both sexes;
Both sexes for angiosarcoma, although more pronounced in the ♂
(f) whether responses are in a single species or several species;
clear carcinogenic effect in the rat, not in the mouse.
(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
None known;
(h) routes of exposure;
Administration of MITC via inhalation
(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;
No evidence of differences between rodents and human
(j) the possibility of a confounding effect of excessive toxicity at test doses;

Nasal tumours observed at doses which induce both corrosion and systemic toxicity. The top-dose may be considered the MTD.

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

The MoA for the observed elevation of nasal tumours is likely associated with the corrosivity of the MITC at the site of contact. It is a top-dose effect displaying a clear threshold. Associated findings in the URT, and more specifically the respiratory/squamous/transitional epithelium are hyperplasia and metaplastic changes (both even observed from the lowest dose onwards), chronic inflammatory changes (from the mid-dose and above) and necrosis of the squamous epithelium, mainly at the top-dose. A secondary induction of the URT tumours subsequent to the corrosive action of MITC (and presumably compensatory regeneration) is therefore plausible.

As explained in the genotoxicity section, MITC is unlikely to be classified as a mutagen, and therefore, MITC is not considered a genotoxic carcinogen. While such a MoA cannot be considered irrelevant for the human per se, the overall picture of the carcinogenic response is not compatible with a typical substance representative of a Carc. Cat 1B.

Overall, RMS consider MITC eligible for classification as Carc Cat 2.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Metam (incl. -sodium and -potassium):
Classified - Carcinogenicity Category 2 – H351: Suspected of causing cancer

MITC:
Classified – Carcinogenicity Category 2 – H351: Suspected of causing cancer

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 56: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Table 56a: Summary of generational toxicity of Metam sodium

Type of test; test species, test substance Metam sodium – batch number – purity; tested doses; Guidance, GLP	Results			References
	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Findings	
Two-generation drinking water, rat (Alpk:APfSD - Wistar-derived) Batch n° BAS/005/00N - Purity: 526 g/L = 43.148% w/w 0, 0.01, 0.03 and 0.10 mg/mL = 0, 1.4, 4.0 and 12.3 mg/kg bw/d (♂) 0, 2.0, 4.9 and 14.4 mg/kg bw/d (♀) Equivalent to OECD 416 (1983) - GLP compliant	Parental 4.0	Parental 12.3	↓body weight during premating, gestation and lactation. Olfactory gland duct: ↑dilatation and hypertrophy with alveolar cell loss and olfactory epithelium degeneration; lymphocytic infiltration in nasolacrimal duct.	[redacted] 1993
	Offspring: 4.0	Offspring: 12.3	↓pup and litter weight	**B.6.6.1.2/01**
	Repro: 12.3	Repro: >12.3	-	

Table 56a1: Litter parameters (including b.w. and food consumption) and reproductive performance 2G study on metam ([redacted], 1993)

Dose (mg/L)	**0**	**0**	**0.01**	**0.01**	**0.03**	**0.03**	**0.1**	**0.1**
Dose (mg/g b.w./d)	**0**	**0**	**1.4**	**1.4**	**4.0**	**4.0**	**12.3**	**12.3**
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight F_0:								
Premating parent							↓2%* wk 3-9	↓3%* wk 2-11
Gestation - d 1,8,22							↓3%*, ↓1%*, ↓8%*	
Lactation - d 1,11,16,22,29							↓6%*, ↓3%*, ↓3%*, ↓6%*, ↓3%*	
Body weight F_1:								
Premating parent							↓4-6%* wk 2-11	↓3-6%* wk 2-11
Gestation - d 1,8,15,22							↓5%*, ↓1%*, ↓2%*, ↓6%*	
Lactation - d 1,5,11,16,22,29							↓11%*, ↓10%*, ↓2%*, ↓4%*, ↓5%*, ↓4%*	
Food consumption:								
F_0 parent premating							↓3-4%* wk 1-5	↓3-4%* wk 1,2,5,6,7,9,10

Dose (mg/L)		**0**	**0**	**0.01**	**0.01**	**0.03**	**0.03**	**0.1**	**0.1**
Dose (mg/g b.w./d)		**0**	**0**	**1.4**	**1.4**	**4.0**	**4.0**	**12.3**	**12.3**
		♂	♀	♂	♀	♂	♀	♂	♀
F_1 parent premating					↓4-5%* wk1,2,4		↓4-5%* wk1,2,4	↓4-7%* wk 1,2,5,6	↓4-7%* wk 1-9
Water consumption: §									
Gestation length (<,=,>22d)°									
F_0 parents	<22	1/30		0/30		0/30		1/30	
	22	29/30		29/30		29/30		29/30	
	>22	0/30		1/30		1/30		1/30	
F_1 parents	<22	0/28		1/30		1/30		1/29	
	22	23/28		24/30		26/30		27/29	
	>22	5/28 *(18%)*		5/30 *(17%)*		3/30 *(10%)*		2/29 *(7%)*	
Fertility:									
F_1A °		29/30	30/30	28/30	30/30	28/30	30/30	30/30	30/30
F_2A °		28/29	29/29	26/30	30/30	26/30	30/30	26/29	29/30
N° pups born live:									
F_1A		341		359		316		348	
F_2A		353		324		361		332	
N° litters all pups born live:									
F_1A		26		24		24		26	
F_2A		20		25		26		22	
Whole litter losses									
F_1A °		1/30		2/30		1/30		0/30	
F_2A °		2/29		0/30		0/30		0/29	
Pup (P) / litter (L) survival:									
F_1A d1-22	P	317		309		296		323	
	L	16		16		17		17	
F_2A d1-22	P	293		284		331		311	
	L	13		16		19		22	
Litter size:									
F_1A	d1	11.7		12.0		10.9		11.6	
	d29	10.9		11.0		10.2		10.6	
F_2A	d1	12.0		10.8		12.0		11.4	
	d29	10.9		9.4		11.0		10.7	
Pup body weight:									
F_1A								↓6-5%* d22-29	↓5%* d29

Dose (mg/L)	**0**	**0**	**0.01**	**0.01**	**0.03**	**0.03**	**0.1**	**0.1**
Dose (mg/g b.w./d)	**0**	**0**	**1.4**	**1.4**	**4.0**	**4.0**	**12.3**	**12.3**
	♂	♀	♂	♀	♂	♀	♂	♀
F_2A							↓8-9% d11-29	↓5-8%* d5-29
Litter weight:								
F_1A	NAD							
F_2A			↓11%*d1 ↓14%*d11 ↓12%*d16 ↓13%*d22				↓11%* d16 ↓14%* d22	
Adjusted litter body weight:								
F_2A							↓8-11%* d5-29	
Pup weight gain (d1-29)**:**								
F_1A							↓5%	↓6%
F_2A							↓10%	↓10%

* Statistically significantly different from control; NAD: no abnormality detected:
°: number of litters with the measured parameter on total number of litters examined.
§ : please refer to separate tables B.6.6.1/01-1 and B.6.6.1/01-4 for water consumption in F_0 and F_1 parental animals, respectively

Table 56b: Summary of reproductive toxicity of MITC

Type of test; test species, test substance MITC – batch number – purity; tested doses; Guidelines, GLP	Results			References, Study n°
	NOAEL	LOAEL	Findings	
Oral (drinking water) two-generation reproductive toxicity study, SD- rat **MITC**, B.n°: 340178; 95.86 – 96.51% Dose levels: 0, 2, 10, or 50 ppm 0, 0.15. 0.71, 3.40 mg/kg bw/d (♂) 0, 0.19, 0.87, 4.22 mg/kg bw/d(♀)OECD 416 (1983) – GLP compliant	Parental: 10 ppm **0.71 mg/kg bw/d**	Parental: 50 ppm 3.4 mg/kg bw/d	↓body weight gain	[redacted] (1987) **6.8.1.5.1/01**
	Offspring: 50 ppm **3.4 mg/kg bw/d**	Offspring: >50 ppm >3.4 mg/kg bw/d	No treatment-related effect *Note: the relevance of 2 litters with 2 pups showing hydrocephaly at the 2 highest doses should be further documented by means of HCD.*	
	Reproductive: 50 ppm **3.4 mg/kg bw/d**	Reproductive: >50 ppm >3.4 mg/kg bw/d	No treatment-related effect	
Inhalation two-generation reproductive toxicity study, SD- rat –full study **MITC,** B. n°: 56198PJV; 99.7% Dose levels: 0, 1, 5, 20 ppm 0, 3, 15, 60 mg/m³ 0, 0.8, 4.0, 16 mg/kg bw/d OECD 416 (2001) – GLP compliant	Parental: 5 ppm 15 mg/m³ **4.0 mg/kg bw/d**	Parental: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↑clinical signs, ↓body weight (gain), ↓food consumption and efficiency, ↑adrenal w., ↓thymus w.; lung: ↑epithelial regeneration, fibrosis, inflammation, ulceration (F_0,F_1); brain: ↑olfactory bulb atrophy* (F_0,F_1) *(*dose w/o effect yet undetermined)*	[redacted] (2014, main study) **B.6.8.4.5.1/02**
	Offspring: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	Offspring: >20 ppm >60 mg/m³ >16 mg/kg bw/d	No treatment-related effect	
	Reproductive: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	Reproductive: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↑oestrous cycle length (F_0,F_1); ↑gestation length (F_0,F_1); ↑vaginal patency delay (F_1); ↑primordial follicle counts (F_1)* *(*dose w/o effect yet undetermined)*	
Inhalation one-generation reproductive toxicity study, SD- rat –range-finding study **MITC,** B. n°: 56198PJV; 99.7% Dose levels: 0, 5, 10, 20 ppm 0, 15, 30, 60 mg/m³ 0, 4.05, 8.0, 16 mg/kg bw/d OECD 421 (1995) – GLP compliant	Parental: 10 ppm 30 mg/m³ **8.0 mg/kg bw/d**	Parental: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↓body weight (gain), ↓food consumption	[redacted] (2013a, RF study) **B.6.8.4.5.1/03**
	Offspring: 10 ppm 30 mg/m³ **8.0 mg/kg bw/d**	Offspring: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↓body weight (gain) during pre-weaning period (F_1).	
	Reproductive: 20 ppm 60 mg/m³	Reproductive: >20 ppm >60 mg/m³	No treatment-related effect	

Type of test; test species, test substance MITC – batch number – purity; tested doses; Guidelines, GLP	Results			References, Study n°
	NOAEL	LOAEL	Findings	
	16 mg/kg bw/d	>16 mg/kg bw/d		
	Developmental: 5 ppm 15 mg/m³ **1.1 mg/kg bw/d**	Developmental: 15 ppm 45 mg/m³ 3.5 mg/kg bw/d	Very slightly altered litter parameters: ↑♂/♀ ratio, ↓foetal viability, ↑early resorption, ↑post implantation loss; single developmental findings (omphalocoele, vertebral centra anomaly, small spleen, 7th sternebra, irregular ossification of 6th sternebra.	

Table 57: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 58: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

METAM sodium

The study provided in this section has already been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. No new study was provided. As data requirements have been adapted since the Annex I inclusion for metam, a re-evaluation of the study was performed.

Two-generation study in rat ([redacted], 1993, **B.6.6.1/01**)
In rats, metam sodium was tested in a multigenerational drinking water study at doses of 0, 1.4, 4.0 and 12.3 mg/kg b.w./d.
At 4 mg/kg bw/d and above, parental body weight was decreased on occasions in both ♂ and ♀ during different phases of the study. Food consumption was low at the top-dose. A dose-dependent decrease of water consumption was observed at all dose-levels.
In top-dose ♀, Bowman's gland duct hypertrophy with loss of alveolar cells was detected in the olfactory mucosa. The changes in the Bowman's gland were accompanied by degeneration/disorganisation/atrophy of the olfactory epithelium. The nasal lesion was a direct consequence of the increased dose taken by ♀ during lactation. Nasal passage of offspring of either generation at any dose level was not affected.
The percentage of live born pups was unaffected in both generations, and there were no litter losses. Both pup weight and weight gain and total litter weight were decreased at the top dose in both generations.
Thus, adverse findings observed in the 2-generation study were restricted to local effects in the upper respiratory tract and reduction of parental and pup weight at the highest dose, indicating general systemic toxicity. One ♀ pup of top dose showed bilateral *anophthalmia*, for which the actual causal relationship with the treatment remains unclear (single findings were also observed in rat developmental studies).
No treatment-related effect on fertility was seen in neither F_0 nor F_1 generation.
Based upon the abovementioned findings both **parental toxicity** and **offspring** NOAEL was 4 mg/kg b.w./d, while the **reproductive** NOAEL was set at the highest dose of 12 mg/kg b.w./d.

MITC

A two-generation study ([redacted], 1986, **B.6.8.1.5.1/01**) was performed on MITC administered orally (in drinking water or via gavage) and has already been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. As further studies with the metabolite MITC have been performed and since the Annex I inclusion for metam the data requirements have been adapted a re-evaluation of the study was performed.
The recent studies om MITC were conducted on MITC administered by inhalation, and include generational studies (two-generation main study, [redacted], 2014, **B.6.8.4.5.1/02**), and a range-finding one-generation study ([redacted], 2013a, **B.6.8.4.5.1/03**).

Information from a reliable two-generation studies in rats exposed to MITC via either oral and inhalational route, showed that MITC has no adverse effects on fertility and reproductive performance of both ♂ and ♀ animals. This is supported by information from the screening study. Affected parameters included gestational and oestrous cycle length, and delayed vaginal patency, which were increased/delayed in the 2-generation study (inhalation), possibly associated with parental toxicity.

In the two-generation rat oral study of [redacted] (1987, **B.6.8.1.5.1/01**), MITC was administered in the drinking water at 2, 10, 50 ppm, corresponding to 0, 0.15, 0.71 or 3.4 mg/kg bw/d. Variations in body weight (gain) were observed in both sexes at the top dose of 3.4 mg/kg bw/d. It is considered that the reduction in body weight gain during premating, gestation and lactation is the most obvious sign of parental toxicity at the top-dose.
A dose-related reduction in water consumption at 0.71 mg/kg bw./d onwards may plausibly reflect the unpalatability of MITC, but in the absence of other adverse effects at this dose, is not considered driving the risk assessment.
Mating performance and fertility were not adversely affected, although fertility and fecundity index, as well as the pregnancy rate, were decreased by 10% at top-dose, in F_0, but not in F_1. Therefore, this modification was not raising particular concern, as the decrease was slight, non-statistically significant and not confirmed in the second generation.
The number of pups born per ♀ was marginally (<6%) lower at 0.71 mg/kg bw./d onwards, however without reaching statistical significance (and only in F_0 but not in F_1). Physical and functional development of pups was comparable amongst all groups. *The occurrence of 2 litters with 2 pups showing hydrocephaly at 0.71 mg/kg bw./d onwards.*
Slight delays in the onset of eye opening and pinna unfolding were observed at top dose but were considered incidental to treatment, as there were no delays in the completion of each parameter, and the findings were not

confirmed in the other generation. Functional development of top dose pups was comparable to controls on d1, d17 and d21.
Overall, MITC was considered devoid of reproductive toxicity (fertility) when administered orally up to about 4 mg/kg b.w./d. In conclusion, the **parental toxicity NOAEL = 10 ppm (0.71 mg/kg bw/d)**, based on decreased bodyweight and body weight gain at the top-dose. Both **reproduction toxicity NOAEL** and **offspring NOAEL** were **> 50 ppm (3.6 mg/kg bw/d**, in the absence of any pup toxicity/reproductive toxicity at the top dose.

In an inhalation multigenerational study ([redacted], 2014) where SD rats were exposed to MITC at 0, 1, 5, and 20 ppm (0, 3, 15, and 60 mg/m³), both F_0 and F_1 parental toxicity was characterised by adverse clinical findings, lower mean (final) body weights and/or lower body weight gains as well as lower mean food consumption/food efficiency generally throughout the study at the top-dose.
MITC-related macroscopic observations in the lungs noted in both parental F_0 and F_1 in the 20 ppm group correlated with microscopic findings at the scheduled necropsy, and included lung epithelial regeneration, fibrosis, inflammation, ulceration in both generations, and brain olfactory bulb atrophy*. Further, there was a higher primordial follicle* count in the ovary (F_1) *(*dose w/o effect not completely determined, and thus to be further confirmed by the notifier).*
There were effects on some F_0 and F_1 reproductive parameters at top-dose level. Reproductive parameters such as oestrous cycle length (F_0,F_1) and gestational length (F_0,F_1), were slightly increased compared to controls.
Other parameters, including the number of days between pairing and coitus, ♂ and ♀ mating and fertility indices, ♂ copulation index, and ♀ conception index were comparable across all groups. Mean sperm motility (including progressive motility), testicular and epididymal sperm concentration, sperm production rate, and the percentage of morphologically normal sperm in the 1, 5, and 20 ppm groups of F_0 and F_1♂ as well as the mean number of implantation sites and unaccounted-for sites in the F_0 and F_1♀ in the same exposure groups were comparable to concurrent control group values.
The mean numbers of F_1 and F_2 pups born, percentage of ♂ at birth, live litter size on PND 0, postnatal survival, and general physical condition of pups in the 1, 5, and 20 ppm groups were unaffected during F_0 and F_1 parental MITC exposure. There were no macroscopic findings noted at any exposure level in the F_1 and F_2 pups that could be attributed to F_0 and F_1 parental test substance exposure. No test substance-related effects were noted on F_1 and F_2 pup organ weights on PND 28 at any exposure level.
Regarding developmental landmarks, the ♂ balanopreputial separation in F_1♂ was comparable across all groups. There was a significant delay in attainment of vaginal patency at top-dose when compared to concurrent controls. Anogenital distance of F_2 animals was not affected by treatment.
Based on the observations made in this inhalation two-generation reproductive toxicity study, the NOAELs were set as follows:
Parental NOAEL= 5 ppm (equivalent to 15 mg/m^3, and **4.05 mg/kg bw/d**), based upon on clinical signs, decreased body weight (gain), food consumption and efficiency, increased adrenal and decreased thymus weights, increased incidences of lung epithelial regeneration, fibrosis, inflammation, ulceration in both generations, and brain olfactory bulb atrophy and a higher primordial follicle count in the ovary (F_1)
Offspring NOAEL= 20 ppm (equivalent to 60.00 mg/m^3, and **16 mg/kg bw/d**), in the absence of adverse findings up to and including the top-dose.
Reprotoxicity NOAEL= 5 ppm (equivalent to 15 mg/m^3, and **4.05 mg/kg bw/d**), based upon increased oestrous cycle length (F_0,F_1); increased gestation length (F_0,F_1), and delayed vaginal patency (F_1).

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

METAM
Not classified.

MITC
Not classified.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 59: Summary table of animal studies on adverse effects on development

Table 59a: Summary of developmental toxicity of Metam sodium

Type of test; test species, test substance Metam sodium – batch number – purity; tested doses; Guidance, GLP	Results			References
	NOAEL (mg/kg bw/d)	**LOAEL (mg/kg bw/d)**	**Findings**	
	Repro: 12.3	Repro: >12.3		
Oral (gavage) developmental study, rat (Chbb, THOM-Wistar-derived, SPF) Batch n° ZH 130585 Purity: 517.3 g/L (42.20%) 0, 10, 40, 120 mg/kg bw/d. 25 animals/dose; Day 6-15 of gestation Equivalent to OECD 414 (1981) - GLP compliant	Maternal: 10	Maternal: 40	↓body weight (gain) and food consumption	[redacted], 1987a **B.6.6.2.1/01**
	Developm.: <10	Developm.: 10	↑dose-related incidence of skeletal variations and retardations (ossification delays). *≥40 mg/kg/d:* ↓foetal weight gain *Top-dose*: 2/1 meningocoele, 1 bilateral microphthalmia.	
Oral (gavage) developmental study, rat (Alpk:APfSD- Wistar-derived, SPF) – range finding study Batch n° BAS/005/00N - Purity: 526 g/L = 43.148% w/w 0, 20, 40, 80 mg/kg bw/d 10 animals/dose; Day 7-16 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Maternal: <20	Maternal: 20	↓body weight gain and food consumption.	[redacted], 1993a **B.6.6.2.1/02**
	Developm.: <20	Developm.: 20	↓foetal weight, ↑post-implantation losses, intra-uterine deaths *Top-dose:* 1 meningocoele	
Oral (gavage) developmental study, rat (Alpk:APfSD (Wistar-derived, SPF) – full study Batch n° BAS/005/00N – 90/2 Purity: 526 g/L = 43.148% w/w 0, 5, 20, 60 mg/kg bw/d 24 animals/dose; Day 7-16 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Maternal: 5	Maternal: 20	↓body weight (gain), ↓food consumption, salivation, stain around mouth, urinary incontinence, vaginal bleeding, kidney pelvic dilatation.	[redacted], 1993b **B.6.6.2.1/03**
	Developm.: <5	Developm.: 5	↑unossified odontoid, cervical vertebrae and calcaneum (variants); a minor defect in a cervical vertebrae center. *≥20 mg/kg/d:* ↓foetal weight, ↑several vertebral column ossification delays. *Top-dose:* ↑post-implantation losses, intra-uterine deaths, 1 unossified cervical arches, 2/2 microphthalmia, 1 anophthalmia, 1 short upper jaw/cleft lip, 3/3 internal hydrocephaly, 1 cerebral meningocoele, ↑*manus/pes* scores (↓ossification).	
Oral (gavage) developmental study, rabbit (NZW) – range-finding studies Batch n° BAS/005/00N – 90/2	Maternal: 5	Maternal: 20	↓faecal output, ↓body weight gain, ↓food consumption *Top-dose:* ↑sloughed mucosa glandular stomach.	[redacted], 1993a-b

Type of test; test species, test substance Metam sodium – batch number – purity; tested doses; Guidance, GLP	Results			References
	NOAEL (mg/kg bw/d)	**LOAEL (mg/kg bw/d)**	**Findings**	
	Repro: 12.3	Repro: >12.3		
Purity: 526 g/L = 43.15% w/w 0, 5, 20, 40, 60 mg/kg bw/d 8 animals/dose; Day 8-20 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Developm.: 20	Developm.: 40	↓foetal weight, ↑post-implantation losses, intra-uterine deaths. *Top-dose:* 1 *cyclopia*	**B.6.6.2.2/01-02**
Oral (gavage) developmental study, rabbit (NZW) – full study Batch n° BAS/005/00N – 90/2 Purity: 525.54 g/L = 43.14% w/w 0, 5, 20, 60 mg/kg bw/d 24 animals/dose; Day 8-20 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Maternal: 5	Maternal: 20	↓body weight gain, ↓food consumption	[redacted], 1993c **B.6.6.2.2/03**
	Developm.: 5	Developm.: 20	↑minor skeletal defects, ↓ossification *Top-dose:* ↓foetal weight, ↑post-implantation losses, intra-uterine deaths, 1 cerebral meningocoele, 2/2 cleft palate	
Oral (gavage) developmental study, rabbit (Himalayan Chbb:HM- outbred) – full study Batch n° ZH 130585 Purity:517.3 g/L = 42.2% w/w 0, 10, 30, 100 mg/kg bw/d 24 animals/dose; Day 6-18 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Maternal: 10	Maternal: 30	↓body weight gain	[redacted], 1987b **B.6.6.2.2/04**
	Developm.: <10	Developm.: 10	↑post-implantation loss, intra-uterine deaths, number of dead implants, ↑visceral variations *≥30 mg/kg/d:* gall bladder agenesis, asymetrical *sternebrae*. *Top-dose:* ↑*truncus arteriosus communis*, 1 *spina bifida*, 1 meningocoele	
Oral (gavage) developmental neurotoxicity study, mouse (Swiss) Batch n° n.d.; formulation used (50% a.s., 510 g/L) 0, 50, 100, 150 mg/kg bw/d 8-10 animals/dose (B. 6.8.2.4/03) 11-18 animals/dose (B. 6.8.2.4/03); GD6-GD21 (B. 6.8.2.4/03) GD6-PND21 (B. 6.8.2.4/04); Deviant from OECD 426 (2018) – not GLP (published study)	Maternal 50	Maternal: 100	mortality	[redacted] 2020 and 2021 (published) **B. 6.8.2.4/03** **B.6.8.2.4./04**
	Developm.: <50	Developm.: 50	limited indications of affected sensimotor functions in young pups and of learning/memory functions in adult offspring	

Remark: numerical indications of structural defects expressed on a foetal (1st number)/litter (2nd number) base.

RMS: at the explicit request of ECHA, RMS replicated the tables of every developmental study used in the abovementioned evaluation.During peer review, tables underneath will not be amended but any adaptation, if appropriate, will be performed on the original tables to be found in Vol.3, B.6.

Table B.6.6.2.1/01 -2: Body weight and b.w. gain of pregnant animals rat developmetal study ([redacted] 1987a).

Dose [mg/kg bw/day] No. of assessed animals	**0** 24	**10** 24	**40** 24	**120** 22
Body weight [g]				
Day of Gestation 0	220.21 ± 8.85	220.42 ± 8.94	220.79 ± 11.37	220 ± 10.35
Δ % of control	-	+0.1	+0.3	-0.1
Day of Gestation 1	221.04 ± 9.47	223.33 ± 9.79	223.92 ± 2.29	223.23 ± 10.31
Δ % of control	-	+1.0	+1.3	+1.0
Day of Gestation 8	250.87 ± 12.14	248.50 ± 13.48	246.92 ± 13.62	**242.77 ± 12.11***
Δ % of control	-	-0.9	-1.6	-3.2
Day of Gestation 13	279.29 ± 13.96	274.67 ± 16.76	271.17 ± 15.74	**265.05 ± 14.59****
Δ % of control	-	-1.7	-2.9	-5.1
Day of Gestation 15	290.33 ± 3.08	286.21 ± 17.69	282.96 ± 15.96	**274.09 ± 14.35****
Δ % of control	-	-1.4	-2.5	-5.6
Day of Gestation 17	312.21 ± 18.32	306.04 ± 21.97	303.12 ± 18.70	**295.05 ± 18.06****
Δ % of control	-	-2.0	-2.9	-5.5
Day of Gestation 20	359.71 ± 24.81	350.21 ± 31.50	346.37 ± 25.44	**336.36 ± 23.14****
Δ % of control	-	-2.6	-3.7	-6.5
Body weight gain [g]				
Day of Gestation 0-1	0.83 ± 7.23	2.92 ± 3.74	3.13 ± 3.25	3.23 ± 3.35
Δ % of control	-	+251.8	+277.1	+289.2
Day of Gestation 6-8	5.50 ± 3.80	4.33 ± 3.87	**-0.42 ± 3.86****	**-4.59 ± 3.87****
Δ % of control	-	**-21.3**	**-107.6**	**-183.5**
Day of Gestation 8-10	10 ± 2.98	9.71 ± 2.90	9.04 ± 3.01	**6.95 ± 3.95****
Δ % of control	-	-2.9	**-9.6**	**-30.5**
Day of Gestation 10-13	18.42 ± 3.83	16.46 ± 5.63	**15.21 ± 4.11***	**15.32 ± 4.25***
Δ % of control	-	**-10.6**	**-17.4**	**-16.8**
Day of Gestation 17-20	47.50 ± 9.29	44.17 ± 11.68	43.25 ± 9.68	41.32 ± 13.03
Δ % of control	-	-7.0	-8.9	**-13.0**
Corrected Body weight [g]				
Day of Gestation 0-20 minus uterus weight	69.38 ± 10.45	64.46 ± 11.05	64.21 ±10.06	**54.32 ± 13.70****
Δ % of control		-7.1	-7.5	**-21.7**

* $p \leq 0.05$; ** $p \leq 0.01$; (Willams test); Δ% = difference to the control in percent

Table B.6.6.2.1/01 -3: Food consumption by mated rats administered metam sodium rat developmetal study ([redacted] 1987a).

Dose [mg/kg bw/day]	**0**	**10**	**40**	**120**
Food consumption [g/day]				
Day of Gestation 0-1	16.3 ± 5.0	17.5 ± 2.0	17.0 ± 1.7	17.5 ± 1.8
Δ % of control	-	+7.4	+4.3	+7.4
Day of Gestation 7-8	21.2 ± 1.6	20.2 ± 1.8	17.8 ± 1.5	17.1 ± 2.0
Δ % of control	-	-4.7	**-16**	**-19.3**
Day of Gestation 11-13	23.9 ± 1.2	23.2 ± 2.3	21.9 ± 1.4	20.3 ± 2.0
Δ % of control	-	-2.9	-8.4	**-15.1**
Day of Gestation 16-17	26.2 ± 1.8	25.3 ± 3.5	25.4 ± 1.1	23.4 ± 3.1

Table B.6.6.2.1/01 -3: Food consumption by mated rats administered metam sodium rat developmetal study ([redacted] 1987a).

Dose [mg/kg bw/day]	**0**	**10**	**40**	**120**
Δ % of control	-	-3.4	-3.1	**-10.7**
Day of Gestation 18-20	27.8 ± 1.7	27.2 ± 3.1	28.1 ± 1.3	26.8 ± 3.6
Δ % of control	-	-2.2	+1.1	-3.6
Day of Gestation 0-20	23.3 ± 1.2	22.8 ± 1.6	22.3 ± 1.0	21.4 ± 1.8
Δ % of control	-	-2.1	-4.3	-8.3

Δ% = difference to the control in percent

Table B.6.6.2.1/01 -4: Summary of litter data rat developmetal study ([redacted] 1987a).

Dose [mg/kg b.w./d]	**0**	**10**	**40**	**120**
Pregnancy status				
No. of total animals	25	25	25	25
No. of pregnant animals	24	24	24	**22**
Rate of conception (%)	96	96	96	**88**
Spontaneous mortalities	0	0	0	0
Dams with abortions	0	0	0	0
Mean Number of				
Corpora lutea	15.21	15.63	14.50	15.05
Implantations	13.75	13.71	12.46	13.95
Dead implantations	1.13	1.96	0.92	2.09
% dead implantations/dam	*7.28*	*17.89**	*6.66*	*14.79**
Live foetuses	12.63	11.75	11.54	11.86
% live foetuses/dam	*92.72*	*82.11**	*93.34*	*85.21**
Mean Weight of (g)				
Uterus	70.13 ± 17.28	65.33 ± 20.10	61.38 ± 19.26	62.05 ± 17.46
Foetuses	3.72 ± 0.20	3.75 ± 0.17	3.60 ± 0.28	**3.42 ± 0.28****
Placenta	0.44 ± 0.04	0.43 ± 0.04	**0.41 ± 0.04* (↓7%)**	**0.41 ± 0.04** (↓7%)**
Proportions of (%):				
♂ foetuses	50.8	46.5	46.6	50.6
Pre-implantation loss	10.37	10.20	15.24	7.34
Post-implantation loss	7.28	17.89*	6.66	**14.79***
No. of resorptions				
early	25	34	19	39
intermediate	2	12	2	5
late	0	0	**1**	**2**
Total	*27*	*46*	*22*	*46*

* $p \leq 0.05$; ** $p \leq 0.01$ (Krauth Test)

Table B.6.6.2.1/01 -5: Summary of external/visceral evaluation rat developmetal study ([redacted], 1987a).

Dose [mg/kg bw/day]	**0**	**10**	**40**	**120**
Caesarean section				
Litters evaluated	***24***	***23***	***24***	***22***
Foetuses evaluated	***303***	***282***	***277***	***261***
Anomalies Foetuses affected # (%) Litters affected # (%)	0	0	0	**2 (0.51)** **1 (4.55)**
Head				
Meningocoele (fœtal/litter incidence)				**2 /1**
Variations # (%) Prop. of litters affected # (%)	0	0	0	0
Retardations # (%) Prop. of litters affected # (%)	0	0	0	0
Organ, Evisceration				
Litters evaluated	***24***	***23***	***24***	***22***
Foetuses evaluated	***204***	***189***	***183***	***173***

Dose [mg/kg bw/day]		**0**	**10**	**40**	**120**
Anomalies Foetuses affected Litters affected	# (%) # (%)	0	0	0	**1 (2.27)** **1 (4.55)**
Head					
Microphthalmia bilateral					**1 /1**
Variations Foetuses affected Litters affected	# (%) # (%)	16 (8.65) 8 (33.33)	28 (**14.75**) 11 (**47.83**)	**72 (39.07)**** **21 (87.50)****	26 (13.04) 11 (50)
Kidneys					
Enlarged renal pelvis bilateral		4	**11**	**29**	14
Enlarged renal pelvis unilateral		12	**17**	**43**	12
Retardations Foetuses affected Litters affected	# (%) # (%)	0	0	0	0
Organ, Barrow-Taylor staining					
Litters evaluated		***24***	***23***	***24***	***22***
Foetuses evaluated		***99***	***93***	***94***	***88***
Anomalies Foetuses affected Litters affected	# (%) # (%)	0	0	0	0
Variations Foetuses affected Litters affected	# (%) # (%)	40 (41.67) 20 (80.33)	31 (32.25) 15 (65.22)	13 (12.78) 7 (29.17)	18 (19.17) 13 (59.09)
Kidneys					
Enlarged renal pelvis bilateral		23	14	5	4
Enlarged renal pelvis unilateral		17	17	8	14
Hydroureter bilateral		3	1		
Hydroureter unilateral	# (%)°	3 (3)	3 (3.2)	1 (1.1)	2 (2.3)
Retardations Prop. of litters affected	# (%) # (%)	0	0	0	0

* p ≤ 0.05; **p ≤ 0.01 (Fischers's exact test, or Krauth asymptotic test)
# = Due to a marginal correction (35 instead of 36 foetuses) this value was calculated later on by interpolation
°: % added by RMS on the basis of incidence/#foetuses evaluated

Table B.6.6.2.1/01 -6: Summary of skeletal evaluation rat developmetal study ([redacted] 1987a).

Dose [mg/kg b.w./d]		**0**	**10**	**40**	**120**
Litters evaluated ***Foetuses evaluated***		***24*** ***204***	***23*** ***189***	***24*** ***183***	***22*** *(↓8%)* ***173*** *(↓15%)*
Anomalies Foetuses affected Litters affected	# (%) # (%)	34 (18.5) 17 (70.8)	44 (22.5) 17 (73.9)	54 (29.8)* 21 (87.5)	35 (21.8)# 17 (77.3)
Sternum					
SNRA (F) *SNRA (%) (HCD: 0.9; 0-2.2)*		0 *0%*	2▪ *1.1%*	12▪▲ *6.5%*	3▪ *1.7%*
SNRA (L)		0	2	11	3
SRA (F)			1	1	1
Vertebral column					
BHCR *BHCR (%) (HCD: 2.1; 0.5-4.7)*		9 *4.4%*	14▲ *7.7%*	17▲ *9.2%*	9▲ *5.2%*
BKHE (F)	# (%)°	21 (10)	23 (12)	24 (13)	20 (12)
BNHR (F)	# (%)°	1 (0.5)	2 (1.1)	4 (2.2)	2 (1.2)
Variations Foetuses affected Litters affected	# (%) # (%)	90 (44.3) 23 (95.8)	97 (50.2) 22 (95.7)	**102 (56.8)*** 24 (100)	**116 (67.3)**** 22 (100)
Ribs					
RBKF (F)		19	27	20	17
RUKF (F)		23	21	15	15
Sternum					
CXG (F)	# (%)°	9	16	19	8
(L)	# (%)°	7 (29.2)	11 (47.8)	11 (45.8)	8 (36.4)
SNV (F)		12	10	0	0
Vertebral column					
BHKU (F)	# (%)°	27 (8.3)	15 (7.9)	28 **(15.0)**	30 **(17.3)**

Dose [mg/kg b.w./d]		**0**	**10**	**40**	**120**
Litters evaluated		***24***	***23***	***24***	***22 (↓8%)***
Foetuses evaluated		***204***	***189***	***183***	***173 (↓15%)***
(L)	# (%)°	14 (58.3)	11 (47.8)	**16 (66.7)**	**17 (72.3)**
BNKU (F)	# (%)°	17	**22▪**	**49▲**	**62▲**
BNKU (%) (HCD: 8.7; 2-14.8)		*8.3%*	***12.2%***	***26.7%***	***35.8%***
BNKU (L)	# (%)°	8 (33.3)	**12 (52.2)**	**19 (79.2)**	**21 (95.5)**
Retardations Foetuses affected	# (%)	152 (75.8)	144 (77.1)	**165 (90.6)****	**158 (91.8)****
Litters affected	# (%)	24 (100)	23 (100)	24 (100)	22 (100)
Head					
HUO (F)	# (%)°	15	9	13	**27 (15.6)**
(L)	# (%)°	12	6	12	**11 (50.0)**
HNO (F)	# (%)°	2 (1.0)	0	2 (1.1)	**5 (2.9)**
(L)	# (%)°	1 (4.2)	0	**2 (8.3)**	**4 (22.7)**
IUO (F)	# (%)°	1 (0.5)	**8 (4.2)**	**20 (10.9)**	**15 (8.7)**
(L)	# (%)°	1 (4.2)	**4 (17.4)**	**11 (45.8)**	**10 (45.5)**
Sternum					
CXZG (F)		38	25	15	20
SNNO (F)		12	7	10	**35▲**
SNNO (%) (HCD : 8.7 ; 4.7-16)		*7.9%*	*4.9%*	*6.1%*	***22.2%***
SNO (F)		34	44	34	31
SNUO (F)	# (%)°	42 (20.6)	25 (13.2)	**57 (31.1)**	**78 (45.1)**
SNUO (L)	# (%)°	18 (75.0)	15 (65.2)	**21 (87.5)**	**21 (95.5)**
SUO (F)		72	75	75	52
Vertebral column					
BNUO (F)					**1**
Pelvic Gridle					
PBU (F)					**3**
Forelimb					
MCBO (F)		0	**1**	**6▲**	**13▲**
MCBO (%) (HCD 0.07 ; 0-0.7)		*0*	*0.7%*	*3.6%*	*8.2%*
MCBO (L)	# (%)°	0	**1 (4.3)**	**4 (16.7)**	**8 (36.4)**
MCUO (F)	# (%)°	0	**3 (1.6)**	**7 (3.8)**	**14 (63.6)**
(L)	# (%)°	0	**3 (13.0)**	**5 (20.8)**	**11 (50.0)**
Hindlimb					
MTBO (F)		**2▲**	**4▲**	**11▲**	**20▲**
MTBO (%)(HCD 0.1 (0-0.7)		*1.3%*	*2.8%*	*6.7%*	*12.7%*
MTBO (L)	# (%)°	2 (8.3)	2 (8.7)	**7 (29.2)**	**10 (45.5)**
MTUO (F)	# (%)°	0	**3 (13.0)**	**7 (29.2)**	**14 (63.6)**
MTUO (L)	# (%)°	0	**3 (13.0)**	**5 (20.8)**	**11 (50.0)**

Statistically significant modification: * $p \leq 0.05$; ** $p \leq 0.01$ (Fischers's exact test, or Krauth asymptotic test);
HCD ▪: above the average value; ▲: above the min-max range; # = Due to a marginal correction (35 instead of 36 foetuses) this value was calculated later on by interpolation; °: % added by RMS, expressed on the basis of foetal incidence/#foetuses evaluated **(F)** or of litter incidence/#litters evaluated **(L)**

Explanation of used acronyms in **table B.6.6.2.1/01 -6:** foetal skeletal evaluation rat ([redacted] 1987a):
CXG = Xiphoid process bipartied
CXZG = Xiphoid process-center bipartied
BHCR = Thoracic vertebra body dumbbell-shaped, notch of the cartilage, cranial
BHKU = Thoracic vertebra body dumbbell-shaped, cartilage unchanged
BKHE = Thoracic vertebra body dumbbell-shaped, notches of the cartilage cranial/caudal
BNHR = Thoracic vertebrae bodies dumbbell-shaped, notches of the cartilage, cranial
BNKU = Thoracic vertebrae bodies dumbbell-shaped, cartilage unchanged
BNUO = Ossification of the thoracic vertebrae bodies incomplete, cartilage present; *alizarin red lightly$^+$/alcian blue$^+$*
HUO = Ossification of the hyoid bone incomplete, cartilage present
HNO = Ossification of the hyoid bone absent, cartilage present ; *alizarin red$^-$/alcian blue$^+$*
IUO = Ossification of the interparietal bone incomplete, cartilage present ; *alizarin red lightly$^+$/alcian blue$^+$*
MCBO = Ossification of the metacarpal bones incomplete, bilateral, cartilage present ; *alizarin red lightly$^+$/alcian blue$^+$*
MCUO = Ossification of the metacarpal bones unilateral incomplete, cartilage present ; *alizarin red lightly$^+$/alcian blue$^+$*
MTBO = Ossification of the metatarsal bones bilateral incomplete, cartilage present ; *alizarin red lightly$^+$/alcian blue$^+$*
PBU = Ossification of the pubic bones bilateral incomplete, cartilage present ; *alizarin red lightly$^+$/alcian blue$^+$*

RBKF = 13-Ribs shortened, cartilages absent, bilateral ; *alizarin red⁺/alcian blue⁻*
RUKF = 13-Ribs shortened, cartilages absent, unilateral ; *alizarin red lightly⁺/alcian blue⁻*
SNNO = Sternebrae not ossified, cartilages present ; *alizarin red⁻/alcian blue⁺*
SNO = Sternebra not ossified, cartilage present; *alizarin red⁻/alcian blue⁺*
SNRA = Sternebra ossification centers dislocated, ventral segments of the ribs asymmetrically fused with the sternum
SNUO = Ossification of sternebrae incomplete, cartilages present; *alizarin red lightly⁻/alcian blue⁺*
SNV = Sternebrae ossification centers dislocated
SRA = Sternebra ossification centers dislocated, ventral segments of the ribs asymmetrically fused with the sternum
SUO = Ossification of sternebra incomplete, cartilage present ; *alizarin red lightly⁻/alcian blue⁺*

Table B.6.6.2.1/02 -2 Body weight and b.w. gain of mated ♀ rat developmental range finding ([redacted], 1993a).

Dose[mg/kg b.w./d]	0	20	40	80
Body weight [g]				
Day of Gestation 1	243.8 ± 17.2	251.9 ± 18.4	245.2 ± 13.9	248.2 ± 19.5
Δ % of control	-	+3.3	+0.6	+1.8
Day of Gestation 4	263.4 ± 21.0	274.5 ± 19.4	265.6 ± 14.7	269.9 ± 20.2
Δ % of control	-	+4.2	+0.8	+2.5
Day of Gestation 7	277.9 ± 20.0	287.3 ± 20.0	277.0 ± 13.9	283.7 ± 20.6
Δ % of control	-	+3.4	-0.3	+2.1
Day of Gestation 10	290.9 ± 21.3	287.9 ± 19.2	278.9 ± 16.8	**270.4 ± 17.3***
Δ % of control	-	-1.0	-4.1	-7.0
Day of Gestation 12	301.7 ± 22.9	295.3 ± 19.7	286.3 ± 18.2	**278.9 ± 18.0***
Δ % of control	-	-2.1	-5.1	-7.6
Day of Gestation 16	325.1 ± 25.8	311.7 ± 17.1	306.3 ± 20.1	**299.8 ± 17.3***
Δ % of control	-	-4.1	-5.8	-7.8
Day of Gestation 22	389.7 ± 33.6	372.3 ± 24.9	369.8 ± 24.0	372.4 ± 25.8
Δ % of control	-	-4.5	-5.1	-4.4
Body weight gain [g]				
Day of Gestation 1-4	19.6 ± 6.8	22.6 ± 5.5	20.4 ± 3.8	21.7 ± 4.4
Δ % of control	-	+15.3	+4.1	+10.7
Day of Gestation 7-10	13.0 ± 3.5	**0.6 ± 3.1****	**1.9 ± 5.5****	**-13.3 ± 6.2****
Δ % of control	-	-95.4	-85.4	-200.8
Day of Gestation 13-16	19.7 ± 5.1	**13.5 ± 4.1****	**15.1 ± 3.5***	16.8 ± 4.6
Δ % of control	-	-31.5	-23.4	-14.7
Day of Gestation 7-16	47.2 ± 7.3	**24.4 ± 6.9****	**29.3 ± 9.4****	**16.1 ± 6.3****
Δ % of control	-	-48.3	-37.9	-65.9
Day of Gestation 19-22	32.7 ± 7.5	26.8 ± 16.7	31.5 ± 9.4	**42.8 ± 8.9***
Δ % of control	-	-18.0	-3.7	+30.9
Day of Gestation 1-22	145.9 ± 22.0	**120.4 ± 16.4***	**124.6 ± 13.0***	**124.2 ± 12.7***
Δ % of control	-	-17.5	-14.6	-14.9

Values (g) expressed in average± s.d.; * $p \leq 0.05$; **$p \leq 0.01$ (Student's t- test, two-sided);
Δ% = difference to the control in percent

Table B.6.6.2.1/02 -3 Food consumption by mated rats administered metam rat developmental range finding ([redacted], 1993a).

Dose [mg/kg bw/day]	**0**	**20**	**40**	**80**
Day of Gestation 1-4	23.0 ± 2.2	23.1 ± 2.0	22.9 ± 1.7	24.0 ± 2.0
Δ % of control	-	+0.4	-0.4	+4.3
Day of Gestation 7-10	26.9 ± 2.0	**21.5 ± 1.3****	**21.3 ± 3.1****	**17.4 ± 2.9****
Δ % of control	-	-20.1	-20.8	-35.3
Day of Gestation 13-16	31.5 ± 2.3	28.3 ± 4.8	**26.7 ± 3.2****	**25.3 ± 2.5****
Δ % of control	-	-10.2	-15.2	-19.7
Day of Gestation 7-16	29.1 ± 2.0	**24.9 ± 2.1****	**24.5 ± 3.0****	**21.8 ± 1.9****
Δ % of control	-	-14.4	-15.8	-25.1
Day of Gestation 16-19	31.9 ± 2.1	29.2 ± 4.8	**28.9 ± 2.5***	**28.3 ± 2.3****
Δ % of control		-8.5	-9.4	-11.3
Day of Gestation 1-22	28.1 ± 1.6	**25.5 ± 2.2****	**25.3 ± 2.2****	**24.7 ± 1.2****
Δ % of control	-	-9.3	-10.0	-12.1

Values (g/animal/d) expressed in average± s.d.; * $p \leq 0.05$; **$p \leq 0.01$ (Student's t- test, two-sided);
Δ% = difference to the control in percent

Table B.6.6.2.1/02 -4 Maternal macroscopic findings administered metam sodium rat developmental range finding ([redacted], 1993a).

Dose [mg/kg b.w./d]	**0**	**20**	**40**	**80**
Animals examined	10	10	10	10
Kidney: Pelvic dilatation	0	1	1	0
Liver: Pale	0	1	0	0
Stomach: raised area/s non-glandular region	0	0	0	**3**

Table B.6.6.2.1/02 -5 Summary of litter data and foetal assessment rat developmental range finding ([redacted], 1993a).

Dose [mg/kg bw/day]	**0**	**20**	**40**	**80**
Parameter				
Number of				
pregnancies	10	10	10	10
♀ with litters	10	10	10	10
intercurrent deaths	0	0	0	0
♀ at term with resorptions only	0	0	0	0
Weight of (g)				
Gravid uterine	84.5 ± 31.2	82.5 ± 11.0	85.3 ± 15.1	77.9 ± 10.4
Litter	60.0 ± 23.7	57.2 ± 8.4	59.0 ± 11.9	52.9 ± 7.5
Foetuses	4.9 ± 0.6	4.5 ± 0.3 **(↓8%)**	4.5 ± 0.2 **(↓8%)**	**4.2 ± 0.3**** **(↓14%)**
Litter mean values:				
Corpora lutea	15.3 ± 1.9	14.2 ± 1.1	14.7 ± 1.7	14.4 ± 1.3
Implantations	13.7 ± 3.2	13.3 ± 1.6	13.4 ± 2.3	13.5 ± 1.1
Pre-implantation losses (%)	10.5	6.3	8.8	6.3
No. of foetuses	12.0 ± 4.8	12.7 ± 2.1	13.1 ± 3.1	12.7 ± 1.3
Post-implantation losses (%)	12.4	**4.5**	**2.2**	**5.9**
Intra-uterine deaths				
early	1.7 ± 1.8	0.6 ± 0.7	**0.3 ± 0.7***	0.7 ± 0.7
late	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
total	*1.7 ± 1.8*	*0.6 ± 0.7*	***0.3 ± 0.7****	*0.8 ± 0.8*
Foetal assessment				
Head: meningocoele	0	0	0	**1**
Hindlimb: Malrotated	1	0	0	0

* $p \leq 0.05$; **$p \leq 0.01$ (Student's t- test, two-sided)

Table B.6.6.2.1/03 -2 Incidence of clinical signs rat developmental study [redacted], 1993b.

Dose level [mg/kg bw/d]	**0**	**5**	**20**	**60**
Animals examined	*24*	*24*	*24*	*24*
Died				
Dry sores 1 or more areas			1	
Discharge from eye				**3**
Eye pallor				**1**
Fore limb damaged		1		1
Hair loss (general)			1	
Pale				**1**
Piloerection				**10**
Salivation			**10**	**24**
Scabs 1 or more areas	1	3	1	1
Signs of diarrhoea	1			
Subacute mass 1/more areas				**5**
Stained around mouth			**5**	**23**
Stained around nose		1	1	**2**
Subdued				**6**
Signs of urinary incontinence			**1**	**10**
Tail damaged		1	1	
Teeth missing				**1**
Vaginal bleeding		1	**3**	**3**
Wet sores 1 or more areas			1	

Table B.6.6.2.1/03 -3 Body weight of mated ♀ rat developmental study [redacted], 1993b.

Dose [mg/kg b.w./d]	**0**	**5**	**20[1)]**	**60[2)]**
Day of Gestation 1	259.5 ± 20.5	258 ± 16.6	252.5 ± 28.4	252.6 ± 16.6
Δ % of control	-	-0.6	-2.8	-2.7
Day of Gestation 4	277.9 ± 20.5	275.3 ± 15.4	278.5 ± 19.6	272.3 ± 13.0
Δ % of control	-	-0.9	+0.2	-2.0
Day of Gestation 7	292 ± 20.0	288.9 ± 15.9	290.8 ± 19.8	284.4 ± 13.7
Δ % of control	-	-1.1	-0.4	-2.6
Day of Gestation 10	305.2 ± 22.0	287.9 ± 19.2	278.9 ± 16.8	**270.4 ± 17.3***
Δ % of control	-	-5.7	-8.6	**-11.4**
Day of Gestation 12	317.4 ± 22.9	**309.6 ± 17.3****	**304.4 ± 20.5****	**286.8 ± 14.0****
Δ % of control	-	-2.5	-4.1	-9.6
Day of Gestation 16	342.9 ± 25.5	**333.8 ± 19.6***	**326.7 ± 23.1****	**307.3 ± 14.5****
Δ % of control	-	-2.7	-4.7	-10.4
Day of Gestation 22	418.8 ± 35.5	414.4 ± 25.0	**405.4 ± 28.6***	**385.0 ± 20.3****
Δ % of control	-	-1.1	-3.2	-8.1

Data expressed in average b.w. (g) ± s.d.; * p ≤ 0.05; **p ≤ 0.01; 1) n = 23 2) n = 21; Δ% = difference to the control

Table B.6.6.2.1/03 -4 Food consumption of dams rat developmental study [redacted], 1993b.

Dose [mg/kg bw/day]	**0**	**5**	**20[1)]**	**60[2)]**
Food consumption [g/day]				
Day of Gestation 1-4	23.7 ± 3.2	23.9 ± 2.0	25.1 ± 1.7	23.8 ± 2.6
Δ % of control	-	+0.8	+5.9	+0.4
Day of Gestation 7-10	29.1 ± 3.2	**27 ± 3.1****	**25.2 ± 2.6****	**19.2 ± 2.5****
Δ % of control	-	-7.2	**-13.4**	**-34.0**
Day of Gestation 13-16	33.5 ± 3.1	**31.4 ± 2.7***	**29 ± 2.5****	**25.8 ± 2.6****
Δ % of control	-	-6.3	**-13.4**	**-23.0**
Day of Gestation 16-19	35 ± 3.0	34.5± 3.7	33.5 ± 2.7	**29.5 ± 2.7****
Δ % of control	-	-1.4	-4.3	**-15.7**
Day of Gestation 19-22	34.9 ± 2.9	34.2 ± 3.2	**32.9 ± 3.3***	**31 ± 3.5****
Δ % of control	-	-2.0	-5.7	**-11.2**

* p ≤ 0.05; **p ≤ 0.01; 1) n = 23 2) n = 21; Δ% = difference to the control in percent

Table B.6.6.2.1/03 -5 Macroscopic findings in dams rat developmental study [redacted], 1993b.

Dose [mg/kg bw/day]	**0**	**5**	**20**	**60**
Animals examined	*24*	*24*	*24*	*24*
Kidney				
Pelvic dilatation	0	0	**1**	**4**
Pale	0	0	0	**1**
Lung : Red area/s	1	0	1	0
Ovary : Mass	0	1	0	0
Ureter : distended	0	0	1	0

Table B.6.6.2.1/03 -6 Litter data rat developmental study [redacted], 1993b.

Dose [mg/kg bw/day]	**0**	**5**	**20[1)]**	**60[2)]**
Litters examined	*24*	*24*	*23*	*21*
Mean Number of				
Corpora lutea	14.4 ± 1.9	14.6 ± 1.8	14.7 ± 1.8	15.0 ± 1.8
Implantations	11.6 ± 3.2	12.2 ± 2.9	12.7 ± 2.9	**13.5 ± 2.6***
Live foetuses	10.9 ± 3.5	11.4 ± 3.3	12 ± 3.2	12.4 ± 3.0
Mean Weight of (g)				
Gravid uterine	78.1 ± 22.5	80.6 ± 19.9	82.0 ± 18.7	78.6 ± 17.7
Litter	54.8 ± 17.1	56.6 ± 14.8	57.1 ± 13.9	53.8 ± 12.8
Foetuses	5.07 ± 0.29	5.03 ± 0.36	**4.8 ± 0.37** (↓5%)**	**4.33 ± 0.22** (↓15%)**
Proportions of (%):				
♂ foetuses	58.8 ± 16.3	52.1 ± 15	50.3 ± 14.2	52.1 ± 14.8
Pre-implantation losses	20.4 ± 17.1	16.8 ± 16.4	13.9 ± 16.1	10.4 ± 12.3*
Post-implantation losses	6.8 ± 9.5	7.6 ± 9.3	5.9 ± 9.4	**8.7 ± 9.2**
Intra-uterine deaths (%)				
early	5.4 ± 9.2	6.0 ± 8.4	5.2 ± 8.5	**6.9 ± 8.6**
Late	1.3 ± 3.9	1.6 ± 6.0	0.7 ± 2.5	1.8 ± 4

* $p \leq 0.05$; ** $p \leq 0.01$; [1)] N = 23 [2)] N = 21

Table B.6.6.2.1/03 -7 Summary of external/visceral evaluation rat developmetal study ([redacted], 1993b).

Dose [mg/kg bw/day]	**0**	**5**	**20**	**60**
Litters evaluated	***24***	***24***	***23***	***21***
Foetuses evaluated	***261***	***273***	***276***	***261***
MAJOR EXTERNAL/ VISCERAL DEFECTS	0.5 ± 2.3	0 ± 0	0.3 ± 1.5	1.8 ± 4.0
Prop. of litters affected	1/24	0/24	1/23	**4**/21
Eyes				
Microphthalmia				
Foetuses : HCD: 0-0.4%	1 (0.4%)			**2 (0.8%)▲**
Litter : HCD : 0-4.5%	1/24 (4.2%)			**2**/21 (9.5%)▲
Anophthalmia				
Foetuses : HCD: 0-0.4%				**1 (0.4%)**
Litter : HCD : 0-4.5%				1/21 (4.2%)
Brain				
Internal hydrocephaly				
Foetuses : HCD: 0-0.7%				**3** (1.1%)▲
Litters : HCD: 0-8.7%				**3**/21 (14.3%)▲
Head				
Exencephaly, lower jaw shortened, umbilical hernia				
Foetuses : HCD: 0-0.4%			1 (0.4%)	
Litters : HCD : 0-4.5%			1/23 (4.3%)	
Meningocoele ; HCD: 0%				**1 (0.4%)▲**
Gross malformation of the skull				
Upper jaw shortened, cleft lip				**1 (0.4%)**
MINOR EXTERNAL/VISCERAL DEFECTS	2.1 ± 7.2	2.2 ± 5.5	0.6 ± 3.0	1.2 ± 3.2
Prop. of litters affected	2/24	4/24	1/23	3/21
Liver				

Table B.6.6.2.1/03 -7 Summary of external/visceral evaluation rat developmetal study ([redacted], 1993b).

Cyst(s) attached		2 (0.7%)	1 (0.4%)	1 (0.4%)
Discoloured area(s)				1 (0.4%)
Ureter : slightly dilated	4 (1.5%)	3 (1.1%)	0	1 (0.4%)
EXTERNAL/VISCERAL DEFECTS VARIANTS Prop. of litters affected	6.5 ± 13.9 7/24	2.1 ± 5.1 4/24	5.3 ± 10.4 8/23	2.5 ± 8.1 3/21
Ureter : kinked	13 (5%)	6 (2.2%)	15 (5.4%)	6 (2.3%)

HCD : ▲: above the min-max range.

Table B.6.6.2.1/03 -8 Summary of skeletal evaluation rat developmetal study ([redacted], 1993b).

Dose [mg/kg bw/day]	**0**	**5**	**20**	**60**
Litters evaluated ***Foetuses evaluated***	***24*** ***261***	***24*** ***273***	***23*** ***276***	***21*** ***261***
MAJOR SKELETAL DEFECTS Prop. of litters affected	0 ± 0 0/24	0.9 ± 3.3 2/24	0.3 ± 1.5 1/23	1.5 ± 3.8 3/21
Skull				
Abnormal zygomatic arch		2 (0.7%)		1 (0.4%)
Gross Malformation	0	0	1 (0.4%)	1(0.4%)
Cervical Vertebrae				
2nd, 3rd and 4th left cervical arches not ossified	0	0	0	**1 (0.4%)**
MINOR SKELETAL DEFECTS Prop. of litters affected	11.8 ± 10.5 16/24	16.8 ± 16.0 18/24	16.9 ± 10.6 **21/23**	**33.5 ± 19.7**** **19/21**
Skull				
Interparietal	1 (0.4%)	0	0	**2** (0.8%)
Parietals-partially ossified	4 (1.5%)	1 (0.4%)	1 (0.4%)	2 (0.8%)
Cervical Vertebrae				
1st – 6th left cervical arches partially ossified (5th right cervical arch reduced)	0	2 (0.7%)	**3 (1.1%)**	**7 (2.7%)**
Centrum not ossified, 4th – 7th HCD: 0-7.1%	4 (1.5%)	5 (1.8%)	**8 (2.9%)**	**27 (10.3%)**▲**
Thoracic Vertebrae				
Centrum Bipartite, 3rd, 5th, 8th, 10-13th	1 (0.4%)	1 (0.4%)	**3 (1.1%)**	**4 (1.5%)**
Centrum partially ossified, 5th, 10-13th	1 (0.4%)	**3 (1.1%)**	**4 (1.4%)**	**5 (1.9%)**
Sternebrae : not ossified, 5th	2 (0.8%)	2 (0.7%)	**8 (2.9%)**	**10 (3.8%)***
SKELETAL VARIANTS Prop. of litters affected	67.8 ± 20.1 24/24	73.9 ± 19.9 24/24	**83.7 ± 21.7**** 23/23	**98.5 ± 4.0**** 21/21
Odontoid not ossified HCD: 14-36%	24 (9.2%)	**35 (12.8%)**	**56 (20.3%)****	**118 (45.2%)**▲**
Cervical Vertebrae				
Centrum not ossified, 2nd Foetuses: HCD: 17-54% Litters: HCD: 74-95.5%	44 (16.9%) 17/24 (71.0%)	**68 (24.9%)*** 19/24 **(79.2%)**	**109 (39.5%)**** 20/23 **(87.0%)**	**185 (70.9%)**▲** 21/21 **(100%)**
Centrum not ossified, 3rd HCD: 3.6-13.3%	10 (3.8%)	12 (4.4%)	**18 (6.5%)**	**60 (23%)**▲**
Transverse process partially ossified, 7th HCD: 10.5-29.4%	30 (11.5%)	31 (11.4%)	17 (6.2%)*	15 (5.7%)*
Not ossified, ventral tubercle HCD: 2.6-10.2%	10 (3.8%)	12 (4.7%)	**26 (9.4%)***	**22 (8.4%)***
Sternebrae: partially ossified, 5th HCD: 11.3-43.8%	76 (29.1%)	70 (25.6%)	80 (29%)	**107 (41%)****
Calcaneum : not ossified Foetuses : HCD: 39-82% Litters : HCD : 87.5-100%	113 (43.3%) 22/24 (92%)	**155 (56.8%)**** 21/24 (88%)	**186 (67.4%)**** 22/23 **(96%)**	**245 (93.9%)**▲** 21/21 **(100%)**
Manus Scores				
Mean manus score per litter HCD: 2.20-4.36%	4.21 ± 0.25	4.16 ± 0.19	4.24 ± 0.23	**4.54 ± 0.34**▲**
Pes Score				

Dose [mg/kg bw/day]	**0**	**5**	**20**	**60**
Litters evaluated	***24***	***24***	***23***	***21***
Foetuses evaluated	***261***	***273***	***276***	***261***
MAJOR SKELETAL DEFECTS	0 ± 0	0.9 ± 3.3	0.3 ± 1.5	1.5 ± 3.8
Prop. of litters affected	0/24	2/24	1/23	3/21
Skull				
Mean pes score per litter HCD: 2.92-4.98%	4.87 ± 0.16	4.86 ± 0.19	4.91 ± 0.18	**5.00 ± 0.12**▲**

Prop. = Proportion; * p ≤ 0.05; **p ≤ 0.01 (Fishers's exact test, two-sided); HCD : ▲: above the min-max range.

Table B.6.6.2.2/02-1 Summary of MATERNAL data range-finding rabbit developmental study ([redacted] 1993b)

Dose [mg/kg b.w./e]	**0**	**5**	**20**	**40**	**60**
Females on study	***8***	***6***	***7***	***5***	***8***
Females evaluated°°	***8***	***6***	***6***	***5***	***8***
Clinical signs: few faeces°	1 (11)	2 (9)	**5 (17)**	**6 (20)**	**7 (27)**
Body weight gain d8-20:		-	**↓8%**	**↓56%**	**↓61%***
Food consumption d8-20:		↓10%	**↓25%**	**↓38%***	**↓46%***
Macroscopic findings:					
Glandular stomach: sloughed mucosa	0	0	0	0	**2**

Table B.6.6.2.2/02-2 Summary of litter data range-finding rabbit developmental study ([redacted], 1993b)

Dose [mg/kg b.w./e]	**0**	**5**	**20**	**40**	**60**
***LITTER** data*					
Litters evaluated	***8***	***6***	***7***	***5***	***8***
Foetuses evaluated	***64***	***56***	***65***	***32***	***44***
Corpora lutea£	10.1 ± 1.5	12.2 ± 1.6*	11.3 ± 2.3	9.8 ± 1.6	11.4 ± 2.3
Implantations£	68 ± 8.5	65 ± 10.8	71 ± 10.1	41 ± 8.2	75 ± 9.4
Live foetuses£	64	56	65	**32**	**44**
Gravid uterine weight£	517.2	625.7	594.4	**413.8** (↓21%)	**347.1*** (↓33%)
Litter weight£	354.9	410.7	405.2	**269.1** (↓26%)	**213.4*** (↓40%)
Fœtal weight £	44.8	44.4	43.9	**41.9** (↓6%)	**40.0 (↓11%)**
Proportions of (%):					
Pre-implantation losses	16.0	11.0	10.1	16.3	17.6
Post-implantation losses	5.9	13.8	8.5	**22.0**	**41.3**
Intra-uterine deaths £					
Early	0.4	0.5	0.9	**1.6**	**3.4***
Late	0.1	1.0*	0.0	0.2	0.5
Total	*0.5*	*1.5*	*0.9*	***1.8***	***3.9****
Anomalies					
Cyclopia					**1/1**
Spina bifida meningocoele	1/1	1/1			

£: average value; * p ≤ 0.05; **p ≤ 0.01

Table B.6.6.2.2/03-1 Summary of pregnancy data rabbit developmental study ([redacted], 1993c).

Dose [mg/kg b.w./d]	**0**	**5**	**20**	**60**
Inseminated	**20**	**20**	**20**	**20**
Not pregnant	0	3	2	1
Intercurrent deaths	0	0	0	0
Pregnant	20	17	18	19
Intercurrent deaths	0	1	1	1
Aborted	0	0	0	**1**
Totally resorbed	0	1	0	0
Live foetuses *in utero*	0	0	1	0
Totally resorbed at termination	0	0	0	**9****
Live foetuses *in utero* at termination	20	16	17	**9****

* p ≤ 0.05; **p ≤ 0.01

Table B.6.6.2.2/03-2: Selected clinical maternal observations rabbit developmental study ([redacted], 1993c).

Dose (mg/kg b.w./d)		0	5	20	60
Blood on tray	# observations	3	3	-	**13**
	# animals	2	2	-	**6**
Few faeces on tray	# observations	2	-	-	**19**
	# animals	2	-	-	**10**
Staining on genital area	# observations	2	-	**5**	**8**
	# animals	1	-	1	**3**
Orange/red stain on tray	# observations	1	-	-	**16**
	# animals	1	-	-	**7**

Table B.6.6.2.2/03-3: Body weight of mated ♀ rabbit developmental study ([redacted], 1993c).

Time point (Days)	Dose Level (mg/kg bw/day)							
	0		5		20		60	
Pre-Dose *(% Control)*								
4	3753	-	3748	*-0.13*	3743	*-0.27*	3781	*+0.75*
8	3844	-	3854	*+0.26*	3838	*-0.16*	3897	*+1.4*
During Dosing *(% Control)*								
9	3866	-	3881	*+0.39*	**3810***	*-1.4*	**3748****	*-3.1*
10	3880	-	3891	*+0.28*	**3827***	*-1.4*	**3662****	*-5.6*
11	3907		3917	*+0.26*	**3837****	*-1.8*	**3657****	*-6.4*
12	3937		3944	*+0.18*	**3858****	*-2.0*	**3685****	*-6.4*
13	3953	-	3954	*+0.03*	**3886***	*-1.7*	**3746****	*-5.2*
14	3971		3996	*+0.63*	3914	*-1.4*	**3769****	*-5.1*
15	4011	-	4039	*+0.70*	3962	*-1.2*	**3874****	*-3.4*
16	4063		4092	*+0.74*	4017	*-1.1*	**3909****	*-3.8*
17	4099		1034	*+0.85*	4050	*-1.2*	**3935****	*-4.0*
18	4110		4141	*+0.75*	4060	*-1.2*	**3955****	*-3.8*
19	4136		4151	*+0.36*	4064	*-1.7*	**3964****	*-4.2*
20	5154		4172	*+0.43*	4060*	*-2.3*	**3975****	*-4.3*
B.w.g. **d9-20** (g)	1288		**291**		**250**		**227**	
Post-Dose *(% Control)*								
23	4243	-	4259	*+0.38*	4138*	*-2.5*	4014**	*-5.4*
26	4336		4341	*+0.12*	4235*	*-2.3*	4104**	*-5.4*
30	4444		4424	*-0.45*	4333	*-2.5*	4199**	*-5.5*

* p = < 0.05, ** p = < 0.01

Table B.6.6.2.2/03-4: Group mean maternal food consumption rabbit developmental study ([redacted], 1993c).

Time point (Days)	Dose Level (mg/kg bw/day)							
	0		5		20		60	
Pre-Dose *(% Control)*								
4 - 8	173	-	186	*+9.2*	178	*+2.9*	200	
During Dosing *(% Control)*								
8 - 11	189	-	189	*0.0*	**154***	***-18.5***	**56****	*-70.4*
11 - 14	178	-	178	*0.0*	170	*-4.5*	**133***	*-25.3*
14 - 17	180	-	174	*-3.3*	173	*-3.9*	**137***	*-23.9*
17 - 20	205	-	198	*-3.4*	**170****	***-17.1***	**147****	*-28.3*
Post-Dose *(% Control)*								
20 - 23	200	-	190	*-5.0*	179	***-10.5***	178	***-11.0***
23 - 26	178	-	170	*-4.5*	161	*-9.6*	185	*+3.9*
26 - 30	156		131**	*-16.0*	136	*-12.8*	171	*+9.6*

* p = < 0.05, ** p = < 0.01

Table B.6.6.2.2/03-5: Summary of litter data rabbit developmental study ([redacted], 1993c).

Dose [mg/kg b.w./d]	0	5	20	60
Mean Number of				
Corpora lutea	11.9 ± 1.5	11.6 ± 2.0	10.8 ± 2.1	11.0 ± 1.0
Implantations	9.45 ± 3.17	10.31 ± 2.77	9.35 ± 2.42	9.67 ± 1.58
Live foetuses	8.40 ± 3.05	9.25 ± 2.98	8.71 ± 2.39	**5.78 ± 2.82**
Mean Weight of (g)				
Gravid uterine	536	565	538	**371***
Litter	364	379	366	**232***
Foetuses	44.8 ± 5.1	42.7 ± 6.6	42.8 ± 4.8	**40.1 ± 4.6***
Proportions of (%):				
♂ foetuses	44.0 ± 16.4	50.2 ± 21.4	59.1 ± 17.9**	**29.7 ± 31.2***
Pre-implantation losses	21.6 ± 23.2	11.2 ± 18.2*	13.6 ± 13.3	12.3 ± 11.9
Post-implantation losses	10.4 ± 13.3	12.6 ± 17.6	6.8 ± 10.4	**41.3 ± 25.6****
Intra-uterine deaths (%)				
Early	8.7 ± 13.3	8.9 ± 16.7	5.2 ± 9.0	**38.2 ± 26.3****
Late	1.7 ± 4.3	3.7 ± 7.0	1.6 ± 3.6	3.1 ± 4.7

* $p \leq 0.05$; ** $p \leq 0.01$

Table B.6.6.2.2/03-6: Summary of MAJOR foetal defects rabbit developmental study ([redacted] 1993c).

Dose [mg/kg b.w./d]	0	5	20	60
Litters evaluated	***20***	***16***	***17***	***9 (↓55%)***
Foetuses evaluated	***168***	***148***	***148***	***52 (↓69%)***
Major external/visceral defects	3.1 ± 7.9	2.2 ± 4.7	0 ± 0	9.0 ± 16.9
Prop. of litters affected	4/20	3/16	0/17	3/9
Head				
Head - Exencephaly		1 (0.7%)		
Head - Meningocoele				
HCD: 0-1.1% (foetal incidence)	0	0	0	**1** (3.8%)▲
HCD: 0-8.3% (litter incidence)	0	0	0	**1** (5.5%)
Cleft palate				
HCD: 0-0.7% (foetal incidence)	0	1 (0.7%)	0	**2** (7.7%)▲
HCD: 0-5.9% (litter incidence)	0	1 (6.3%)▲	0	**2** (11.1%)▲
Brain – internal hydrocephaly	1	0	0	0
Spina bifida – meningocoele	1	0	0	0
Hindlimb malrotated	2	1	0	1
Hindlimb malformed	0	0	0	**1**
Skull gross malformation	0	0	0	**1**
Zygomatic arch abnormal	1	0	0	0
Vertebral column major defect	1	0	0	0
Sacral vertebra malformed	0	0	0	**1**
Caudal vertebrae absent	0	0	0	**1**
Fused /absent ribs	0	0	**2**	0
Pelvic girdle	0	0	0	**1**

* $p \leq 0.05$; ** $p \leq 0.01$ (Fishers's exact test, two-sided); HCD : ▲: above the min-max range.

Table B.6.6.2.2/03-7: Summary of MINOR foetal defects rabbit developmental study ([redacted], 1993c).

Dose [mg/kg b.w./d]	0	5	20	60
Litters evaluated	***20***	***16***	***17***	***9***
Foetuses evaluated	***168***	***148***	***148***	***52***
Minor external/visceral defects	6.9 ± 12.5	6.4 ± 9.8	3.6 ± 9.2	4.9 ± 11.3
Prop. of litters affected	7/20	6/16	3/17	2/9
External/visceral variants	0	0	0	0
Minor skeletal defects	48.9 ± 19.2	45.9 ± 21.5	42.7 ± 19.6	43.9 ± 33
Prop. of litters affected	20/20	15/16	16/17	8/9
Cervical Vertebrae, transverse process partially ossified 7th	0/0	**3/1**	**6*/3**	1/1
%	***0/0***	***2.0/6.3***	***4.1/17.6***	***[1.9/11.1]***

Table B.6.6.2.2/03-7: Summary of MINOR foetal defects rabbit developmental study ([redacted], 1993c).

Extra rib: 13th, short length and floating	15/9	23/9	13/8	0*/0*
Lumbar vertebrae § transverse processes fully ossified 3rd	10/7	8/5	**5/4**	**0/0**
%	6.0/35.0	5.4/31.3	**3.4/23.5**	**0.0/0.0**
Sternebrae :				
present, 7th	0/0	0/0	1/1	7*/2
not ossified, 5th	22/9	17/6	15/8	0*/0*
partially ossified 2nd §	0/0	0/0	0/0	**2/2**
Skeletal Variants	82.2 ± 15.3	89.7 ± 15.5	**93.2 ± 9***	**98.6 ± 4.2****
Prop. of litters affected	20/20	16/16	17/17	9/9
Odontoid partially ossified	75/16	83/15	75/15	**42*/9**
%	*44.6/80.0*	*56.1/93.8*	*50.7/88.2*	***80.8/100.0***
Vertebral column, 27 presacral vertebrae	34/13	26/9	**50***/13	**37***/9
HCD: 14.6% – 48.5% (foetal)	(20.2%)	(17.6%)	**(33.8%)**	**(71.2%)▲**
Extra rib: 13th, normal length	60/16	49/11	68/17	**45*/9**
Sternebrae, partially ossified, 5th	64/18	54/14	55/15	7**/5

* p ≤ 0.05; **p ≤ 0.01 (Fishers's exact test, two-sided); HCD : ▲: above the min-max range.
[..]: non dose-responsiveness difficult to interpret taking into account the meaningful reduction of number of litters/foetuses at target at the top-dose; §:not formerly reported

Table B.6.6.2.2/04-1: Maternal body weight parameters rabbit developmental study ([redacted], 1987b).

Dose [mg/kg b.w./d]	**0**	**10**	**30**	**100**
Day 0	2192 ± 149	2189 ± 69	2180 ± 115	2202 ± 98
Δ % of control	-	-0.2	-0.6	0.4
Day 18	2309 ± 186	2360 ± 80	2318 ± 136	2322 ± 138
Δ % of control	-	2.2	0.4	0.6
Day 29	2476 ± 176	2557 ± 93	2478 ± 152	2401 ± 164
Δ % of control	-	3.2	0.1	-3.1
Day 25-28	64	65	57	37
Δ % of control	-	1.5	**-11**	**-42**
Day 28-29	21	20	9	6
Δ % of control	-	-5	**-57**	**-71**
Day 0-29	284	368	298	199
Δ % of control	-	30	5	**-30**

* p ≤ 0.05; **p ≤ 0.01; data expressed in average weight± s.d.; Δ% = difference to the control in percent

Table B.6.6.2.2/04-2: Summary of litter and foetal data rabbit developmental study ([redacted], 1987b).

Dose [mg/kgb.w./d]	**0**	**10**	**30**	**100**
Conception rate (%)	100	100	93.3	100
Mean Number of				
Corpora lutea	7.36	7.77	7.93	7.80
Implantations	6.07	7.08	6.00	7.13
Live foetuses/pregnant rabbit (mean #)	5.79	6.54	5.21	**3.20**
Live foetuses (% per animal)	96	92	**88(+)**	**45****
Mean Weight of (g)				
Gravid uterine	301 ± 90	337 ± 87	281 ± 98	**187 ± 108****
Foetuses	39 ± 4	38 ± 3	39 ± 4	40 ± 4
Proportions of (%)				
♂ foetuses	45	39	44	49
Pre-implantation losses	17.59	8.74	23.79	8.84
Post-implantation losses	3.7	**8**	**12***	**55****
Intra-uterine deaths (%)				

Table B.6.6.2.2/04-2: Summary of litter and foetal data rabbit developmental study ([redacted], 1987b).

Dose [mg/kgb.w./d]	**0**	**10**	**30**	**100**
Conception rate (%)	100	100	93.3	100
Mean Number of				
Early	1	**6**	**7**	**40**
Intermediate	2	1	3	**10**
Late	1	0	1	**2**
Total	*4*	***7***	***11***	***52***
Abort	0	0	0	**7**
Dead implants (mean no)	0.29	**0.54**	**0.79**	**3.93**

* $p \leq 0.05$; ** $p \leq 0.01$;(+): trend .

Table B.6.6.2.2/04-3: Foetal evaluation rabbit developmental study ([redacted], 1987b)

Dose [mg/kg b.w./d]	**0**	**10**	**30**	**100**
Litters available	***14***	***13***	***14***	***14***
Foetuses available	***81***	***85***	***73***	***48***
Anomalies (Caesarian section)	0	0	0	**2/14**
Variations (Caesarian section)	0/0	3/15	1/7	**2/7**
Retardations (Caesarian section)	0	0	0	0
Vertebral column *Spina bifida* (foetal) HCD: 0%	0	0	0	**1 (2%)** ▲
Head Meningocoele (foetal) HCD: 0%	0	0	0	**1 (2%)**
Visceral anomalies	0	0	**1/7**	**5/14**
Visceral variations	52/79 (66%)	**39/54 (72%)**	**47/57 (82%)**	**49/57 (86%)**
Visceral retardations	0	0	0	0
Gall bladder agenesis (HCD, foetal: 1.2%)	0	0	**1 (1.4%)**▲	**1 (2.1%)**▲
Truncus arteriosus communis (HCD, foetal: 1.1%) °	0	0	0	**1 (2%)** ▲
Skeletal anomalies	0	0	0	0
Skeletal variations	13/36	6/15	11/29	15/43
Skeletal retardations	69/100	67/100	61/86	30/50
Sternum Asymetrical sternebrae (HCD, foetal: 0.6 - 6%)	1 (1.2%)	2 (2.4%)	**7 (9.6%)** ▲	**6 (12.5%)** ▲

* $p \leq 0.05$; ** $p \leq 0.01$ (Fishers's exact test, two-sided); ▲: above the min-max range.
a / b: foetal incidence/litter incidence [%]; °: referred to as for «anomalous course of blood vessels»

Table 59b: Summary of developmental toxicity of MITC

Type of test; test species, test substance MITC – batch number – purity; tested doses; Guidelines, GLP	Results			References, Study n°
	NOAEL	LOAEL	Findings	
Oral (gavage) developmental toxicity study, Wistar rat, from GD 6–15 **MITC,** B. n° ZH 6205 MK, 96.9% Dose levels: 0, 3, 10, 30 mg/kg bw/d OECD 414 (1981) – GLP compliant	Maternal: 3 mg/kg bw/d	Maternal: 10 mg/kg bw/d	↓body weight gain, ↑water consumption	[redacted], 1987 **B.6.8.1.5.2/01**
	Developmental: **3 mg/kg bw/d**	Developmental: 10 mg/kg bw/d	↓foetal weight, ↓placental weight, ↑runts No adverse developmental findings.	
Inhalation developmental toxicity study, SD- rat, from GD 6–20 **MITC,** B. n°: 56198PJV, 99.7% Dose levels: 0, 1, 4, 12 ppm, 0, 3.12, 12.39, 36.33 mg/m³ 0, 0.8, 3.2, 9.7 mg/kg bw/d OECD 414 (2001) – GLP compliant *Dose levels identified in a rat RF study at 0, 1, 5, 20 ppm; ↓b.w., f.c., foetal w.) at 20 ppm.*	Maternal: 4 ppm 12 mg/m³ **3.2 mg/kg bw/d**	Maternal: 12 ppm 36 mg/m³ 9.7 mg/kg bw/d	↓body weight and body weight gain; ↓food consumption	[redacted] (2012d, main study) **B.6.8.1.5.2/03** [redacted] (2013b, RF study) **B.6.8.1.5.2/02**
	Developmental: 4 ppm 12 mg/m³ **3.2 mg/kg bw/d**	Developmental: 12 ppm 36 mg/m³ 9.7 mg/kg bw/d	↑major blood vessel variation; ↓ossification of the 13th rib	
Oral (gavage) developmental toxicity study, Chinchilla-rabbit, from GD 6 – 18 **MITC,** B. n° ZNT 85/231-2; 98% Dose levels: 0, 1, 3, 10 mg/kg bw/d, OECD 414 (1981) – GLP compliant	Maternal: 3 mg/kg bw/d	Maternal: 10 mg/kg bw/d	↓body weight and body weight gain	[redacted] 1986 **B.6.8.1.5.2/07**
	Developmental: **10 mg/kg bw/d**	Developmental: >10 mg/kg bw/d	No adverse developmental findings.	
Inhalation developmental toxicity study, NZW- rabbits, from GD 7 – 28 **MITC,** B. n°: 56198PJV; 99.7% Dose levels: 0, 1, 5, 15 ppm 0, 3, 15, 45 mg/m³ 0, 0.2, 1.1, 3.5 mg/kg bw/d OECD 414 (2001) – GLP compliant *Dose levels identified in (i) inhalational RF study at 0, 1, 4, 12 ppm; ↓b.w., ↓f.c., ↓foetal w) at 12 ppm and in (ii) a 21d- pilot study at 0, 5, 10, 20, 60 ppm, fatalities at ≥20 ppm, and clinical signs, ↓b.w.c., f.c. at ≥5 ppm.*	Maternal: 5 ppm 15 mg/m³ **1.1 mg/kg bw/d**	Maternal: 15 ppm 45 mg/m³ 3.5 mg/kg bw/d	*At beginning of dosage*:↑clinical signs, ↑mortality, ↑moribundity, ↓body weight. *Further*: ↓body weight, ↓food consumption, ↑maternal macroscopic findings	[redacted] (2012c, main study) **B.6.8.1.5.2/06** [redacted] (2012b, RF study) **B.6.8.1.5.2/05**
	Developmental: 5 ppm 15 mg/m³ **1.1 mg/kg bw/d**	Developmental: 15 ppm 45 mg/m³ 3.5 mg/kg bw/d	Very slightly altered litter parameters: ↑♂/♀ ratio, ↓foetal viability, ↑early resorption, ↑post implantation loss; single developmental findings (omphalocoele, vertebral centra anomaly, small spleen, 7th sternebra, irregular ossification of 6th sternebra.	[redacted] 2012a, 21d-inhalation pilot study) **B.6.8.1.5.2/04**

Table 60: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 61: Summary table of other studies relevant for developmental toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

Metam (incl. -sodium and -potassium):

All studies provided in this section have already been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. No new study was provided. As data requirements have been adapted since the Annex I inclusion for metam, a re-evaluation of the studies was performed.

Developmental studies were performed in rats by gavage at doses ranging from 5 up to 120 mg/kg bw/d ([redacted], 1987, **B.6.6.2.1/01**; [redacted], 1993a-b, **B.6.6.2.1/02-03**).
Maternal toxicity started at 5 mg/kg bw/d ([redacted] 1993b) as suggested by the dose-related reduced body weight (gain), food consumption, clinical signs and kidney pelvic dilatation. Reproduction parameters were not affected.
Significantly increased post-implantation losses and intra-uterine deaths were reported at 20-60 mg/kg bw/d ([redacted] 1993 a,b). At 60 mg/kg bw/d, one ♀ showed complete resorption.
Reduced mean foetal weight was evident at 20 mg/kg bw/d and above.

Major defects were reported at 120 mg/kg bw/d, 2 foetuses/1 litter exhibited a meningocoele (neural tube closure defect) and 1 foetus had bilateral microphthalmia. At 80 mg/kg bw/d, 1 foetus had a meningocoele. At 60 mg/kg bw/d 5 foetuses/5 litters were affected: defects of eyes (3 foetuses with other head defects); 1 foetus had a shortened jaw and cleft lip, 1 had meningocoele, 1 foetus had unossified 2nd, 3rd and 4th arches of the cervical vertebrae, and 1 foetus displayed an abnormal zygomatic arch. At 20 mg/kg, one foetus had 4 major defects, including a shortened lower jaw. None of those has been seen historically in controls in the laboratory.

At 5 mg/kg b.w./d ([redacted] 1993b, see Vol.3, B.6, table B.6.6.2.1/03-8) and 10 mg/kg b.w./d ([redacted] 1987a, see Vol.3, B.6, table B.6.6.2.1/01-6) onwards, dose-dependent variations/retardations were detected in the vertebral column and in sternum and the number affected litters were increased as compared to the control. These effects were assessed as sign of immaturity of the foetuses. Retarded ossification of foetal skeletons, especially the incomplete ossification of skull bones could be connected with the mechanism of development of meningocoele and the implication of the treatment with metam sodium cannot be excluded.
Overall, the rat **maternal NOAEL is 5 mg/kg b.w./d**, while the **developmental NOAEL was <5 mg/kg b.w./d**. RMS acknowledges that overall, most anomalies were noted at 20 mg/kg b.w./d onwards, but the dose-dependent findings at the lowest dose and above cannot be disregarded for the setting of the NOAEL.

In rabbits, metam sodium was administered by gavage at doses ranging from 5 up to 100 mg/kg bw/d ([redacted] 1987b, **B.6.6.2.2/04**; [redacted], 1993a-c, **B.6.6.2.2/01-03**).
Body weight (gain) of dams and food consumption was decreased at 20-30 mg/kg bw/d and above.
The dose at which the number of post-implantation loss, intra-uterine deaths, number of dead implants, and visceral variations was increased in rabbits was 10 mg/kg bw/d onwards ([redacted], 1987b, see Vol.3, B.6 table B.6.6.2.2/04-2). Both post-implantation loss and intra-uterine deaths increased at the higher doses of 40-60 mg/kg b.w./d ([redacted], 1993b,c, see vol.3 B.6 tables B.6.6.2.2/03-5, -6 and -7), along with foetal weight decreases.
Throughout the 3 rabbit developmental studies, an increased incidence of minor skeletal defects and ossification delays at ≥20 mg/kg b.w./d, gall bladder agenesis and asymmetrical sternebrae (≥30 mg/kg b.w./d), single incidences of cyclopia and meningocoele, were observed. At 60 mg/kg b.w./d, 2 litters carrying cleft palate occurred. At 100

mg/kg b.w./d, a single t*runcus arteriosus communis*, 1 *spina bifida*, and another meningocoele was observed.
Therefore, it was proposed that in rabbit, the overall **maternal NOAEL = 5 mg/kg bw/d**, and that the developmental **NOAEL was also 5 mg/kg b.w./d**, taking into consideration the dose-spacing where the latter value (obtained in the [redacted] study) covers the LOAEL of the [redacted] study.

Conclusions:
Considering the rat full developmental studies together, it is considered that the administration of metam sodium via gavage was associated with increased incidences of minor developmental effects including minor anomalies, skeletal variations and ossification delays (mainly at vertebral column level), occasionally at the dose below that causing maternotoxicity.
At doses at or above maternotoxicity, single severe craniofacial malformations including meningocoele and microphthalmia, but also internal hydrocephalus in 3 foetuses among 3 litters. Especially meningocoele occurred in a consistent way, including in the rat range-finding study.

In the first rabbit (Himalayan strain) full developmental study ([redacted] 1987b), the setting of the developmental NOAEL was based on increased post-implantation loss, intra-uterine deaths and number of dead implants, observed at a dose of 10 mg/kg b.w./d, which caused no or minimal maternal toxicity. The post-implantation losses and intra-uterine deaths in the full studies corroborated the finding s in a range-finding study. It was observed that these findings were replicated in the second full study on NZW rabbits ([redacted] 1993c) but this time at the top-dose, exceeding by 3× the maternally toxic dose of 20 mg/kg b.w./d.
It was remarkable that meningocoele was observed in the rabbit, like in the rat, in singularity at the top-dose.
Other major (but rare) structural findings at the top-doses included single occurrences of cyclopia and *spina bifida*. Two litters showed 1 foetus each with cleft palate. Minor skeletal defects and ossification retardations were observed either below (first study) or at/above maternally toxic doses.

Based upon the former and the current peer-review, a WoE approach is considered appropriate to propose a classification of metam sodium for developmental toxicity. Both in the rat and in the rabbit, developmental effects are observed at doses below maternal toxicity. In the case of the rat, these effects are relatively mild (ossification delays, variants), while in the rabbit more severe foetal toxicity (including post-implantation loss and intra-uterine death) below maternotoxicity in one on the two conducted studies. In the other study, these findings are observed at overt maternotoxic doses.
Regarding structural defects, including craniofacial malformations, the findings are observed at top-doses exceeding maternal toxicity. On the other hand, one major and rare malformation, meningiocoele, is quite consistently found at low incidence in both the rat and the rabbit.

On balance, RMS would propose to allocate the classification **Repr.2, H361d «*Suspected of damaging the unborn child*»** to the a.s. metam sodium, taking into consideration the severity of the major structural defects, albeit at low incidence at the top-doses, exceeding maternotoxicity in one out of 2 studies, considering also the presence of minor anomalies, variants and skeletal ossification delays.

MITC

Existing studies were performed on MITC administered orally (in drinking water or via gavage), and comprise a two-generation study ([redacted] 1986, **B.6.8.1.5.1/01**) as well as developmental toxicity studies in rat ([redacted] 1987, **B.6.8.1.5.2/01**) and rabbit ([redacted]1986, **B.6.8.1.5.2/07**), which already have been evaluated during Annex 1 inclusion and thus peer-reviewed by European competent authorities and Belgium as the rapporteur Member State. As further studies with the metabolite MITC have been performed and since the Annex I inclusion for metam the data requirements have been adapted a re-evaluation of the studies was performed.
The recent studies om MITC were conducted on MITC administered by inhalation, and include generational studies (two-generation main study, [redacted] 2014, **B.6.8.4.5.1/02**), and a range-finding one-generation study ([redacted], 2013a, **B.6.8.4.5.1/03**).
The new developmental studies were performed both in the rat (dose ranging [redacted], 2013b, **B.6.8.1.5.2/02**, main study [redacted] 2012d, **B.6.8.1.5.2/03**) and in the rabbit (two screening studies [redacted], 2012a-b, **B.6.8.1.5.2/04**, **B.6.8.1.5.2/05**, and the main study [redacted], 2012c, **B.6.8.1.5.2/06**).

In the oral rat developmental study ([redacted], 1987, **B.6.8.1.5.2/01**), MITC was administered by gavage at 0, 3, 10 or 30 mg/kg bw/d. At the end of the treatment, several dams at top dose had sticky and/or moist fur in the area of the snout before, and at the same location reddish but mainly dry fur. Body weight and body weight gain were significantly reduced at top dose and marginally at intermediate dose. Water consumption was increased in individual dams at 10 mg/kg bw/d and above.
Reproduction data were not significantly affected in any treated group.
At the top-dose, the number of foetuses weighing <75% of the mean foetal weight/litter was increased, and the placenta weight was significantly lowered.

Sex distribution was comparable in the different test groups.
One anomaly (anophthalmia) was detected in one foetus/one litter at the mid-dose of 10 mg/kg bw/d.
Except for the slightly increased number of runts, observed at the top-dose, there were no significant differences between the treated groups and the controls with regard to structural anomalies, variations and /or retardations.
Based on the observations made in this oral developmental rat toxicity study, the NOAELs were set as follows:
The **maternal NOAEL = 3 mg/kg bw/d,** taking into account the reduced body weight gain of dams and an increased water consumption observed at 10 mg/kg bw/d and above.
The **developmental NOAEL = 10 mg/kg bw/d** based on the reduced placental weight, reduced foetal weight, and foetal stunting observed at the top-dose of 30 mg/kg bw/d.

In the main inhalation rat developmental toxicity study, the animals were exposed to MITC from GD 6–20 at target concentrations of 0, 1, 4 and 12 ppm (equivalent to 0, 3.12, 12.39 and 36.33 mg/m³). Treatment-related effects were characterised by significantly reduced food consumption, reduced body weight and body weight gain at the top-dose of 12 ppm. There were no signs of treatment-related effects on any litter parameters, and foetal weights remained unaffected at any exposure level. However, at the top-dose, subtle modifications were observed including ossification delay at the level of the of the 13th rib (4 litters affected at top-dose). In addition, a single case of a major blood vessel variation (right carotid and right subclavian arteries arose independently from the aortic arch) was observed.
Therefore, the **maternal NOAEL= 4.13 ppm** (equivalent to **12.39 mg/m³** or **3.35 mg/kg bw/d)**, based on ↓body weight (change) and food consumption at the top-dose.
The **developmental NOAEL** was also determined at **4.13 ppm**, based on the increased occurrence of major blood vessel variation and reduced ossification of the 13th rib at top dose.
Overall, the developmental findings are restricted to variations and the limited occurrence of these are deemed of insufficient severity, and moreover possibly linked to maternotoxicity at the same dose-level, justifying non-classification under CLP.

In rabbits ([redacted], 1986, **B.6.8.1.5.2/07**), MITC was given orally by gavage at 1, 3 or 10 mg/kg bw/d. No test article related signs or symptoms were observed in any ♀ of the different groups. At top dose, a slightly higher reduction of mean body weight was considered to be compound-related. Food consumption was reduced at top dose from day 6-11.
No effects were seen on the mean number of implantations, pups or embryonic deaths. Body weight of foetuses were not affected. Sex ratio was similar in all groups. Investigations of the crania and body cavity and skeletal of the fetuses did not show compound-related effects. Thus, the administration of MITC by gavage to pregnant rabbits during organogenesis elicited signs of maternal toxicity at 10 mg/kg bw/d. and there was no effect on the investigated reproduction data at this dose.
A **maternal toxicity NOAEL= 3 mg/kg b.w./d**, and a **developmental toxicity NOAEL = 10 mg/kg b.w./d** is proposed accordingly. MITC was not embryo/foetotoxic and did not elicit abnormal findings in the litters.

In the main inhalation rabbit developmental toxicity study of [redacted] (2012c, **B.6.8.1.5.2/06**), MITC was administered to the animals from GD 7-28 at 0, 1, 5, or 15 ppm (equivalent to about 0, 3, 15, or 45 mg/m³)
Clinical signs of toxicity were evident in ♀ of the 15 ppm top-dose group which were largely characterised by local (portal of entry) effects. The ♀ that died or were euthanised *in extremis* prior to scheduled necropsy were noted with dark red areas/discoloration of the lungs, firm lungs, lungs not fully collapsed, and white or thick white contents of the trachea or stomach. A significantly lower mean food consumption and corresponding significantly reduced mean body weight gains during gestation were also noted in the top-dose ♀. Clinical findings noted in ♀ of the 1 and 5 ppm groups were considered of uncertain toxicological relevance, but the severe toxic findings at the top-dose, observed at the beginning of the dosage, may be of major relevance for the determination of acute reference values. Based on mortality, moribundity, body weight losses, decreased food consumption, and maternal macroscopic findings in the 15 ppm group, the **maternal toxicity NOAEL = 5 ppm (15 mg/m³** or **1.1 mg/kg b.w./d)**.

Subtle effects were noted at 15 ppm on embryo/foetal growth, survival, or external, visceral, and skeletal morphology.
The **developmental NOAEL** was also derived at **5 ppm (15 mg/m³** or **1.1 mg/kg b.w./d)**, based on very slightly altered litter parameters (↑♂/♀ ratio, ↓foetal viability, ↑early resorption, ↑post implantation loss) and single developmental findings (omphalocoele, vertebral centra anomaly, small spleen, 7th sternebra, irregular ossification of 6th sternebra), observed at the top-dose.

Overall, it may be concluded that the administration of MITC to both rat and rabbit, despite the presence of weak developmental or foetotoxic effects, at or above maternal toxicity levels, does not trigger the need for a classification for developmental toxicity. Likewise, no adverse effects are observed which would justify a classification for fertility endpoints (the slightly affected oestrus cycle and gestational lengths had no impact on apical reproductive performance endpoints, and did not qualify for any classification).

Overall, **RMS** considers that MITC is devoid of reprotoxicity hazard, and the slight effects observed at maternotoxic doses are insufficient to propose a classification for reprotoxic toxicity under the CLP regulation.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Metam

As discussed hereabove (**2.6.6.2.1**), On balance, RMS would propose to allocate the classification **Repr.2, H361d «*Suspected of damaging the unborn child*»** to the a.s. metam sodium, taking into consideration the severity of the major structural defects, albeit at low incidence at the top-doses, exceeding maternotoxicity in one out of 2 studies, considering also the presence of minor anomalies, variants and skeletal ossification delays.
RMS proposes however to discuss this issue in an expert consultation.

In order to put together both maternal effects and foetotoxic effects, RMS made an overview of the critical endpoints, in tables 61a and.61b. Reference is also made, for more detail to the sections B.6.6.2.1/01 to -03 (rat developmental studies), and B.6.6.2.2/01 to -04 (rabbit developmental studies.

In these tables, the association is clarified between the doses causing overt maternal toxicity, and the incidences of severe foetal adverse effects (such as litter parameters or structural/visceral alterations), and less severe effects such as body weight effects or minor alterations/variations like ossification delays.

In the rats, severe foetal effects are observed from 40 mg/kg bw/d onwards, and consistently observed at doses which display also overt maternotoxicity. Severe litter effects include embryonic resorption/ death (40, 60 and 120 mg/kg bw/d), post-implantation loss (60 and 120 mg/kg bw/d), and malformations: single incidence of meningocoel (80 and 120 mg/kg bw/d). Structural defects like hydrocephalus (60 mg/kg bw/d, but not above), and microphthalmia (60 and 120 mg/kg bw/d) were notable but lacked dose-dependency when considered throughout the various studies. Less severe foetal effects, including ossification delays showed a dose-dependent pattern in the [redacted]1993c study, in the presence of minimal to strong maternal toxicity.

In the rabbits, embryofoetal toxicity expressed as increased post-implantation loss and uterine death occurred in all studies, along with overt maternal toxicity at the doses of 30, 40, 60 and 100 mg/kg bw/d. A decreased number of live foetuses was found at 40 and 60 mg/kg bw/d. In 1/3 experiments ([redacted] 1987b), this embryofoetal toxicity occurred in the absence of maternal toxicity. In the absence of a clear replication of this outcome in a second valid reprotoxicity study in the rabbit, a clear conclusion as regards a specific embryofoetal toxicity vannot be drawn, though.
Structural defects occurred in singularity, like gall bladder agenesis, cyclopia, and menigocoel) or in 2 foetuses (cleft palate), but for these findings, neither the number of affected litters nor the dose-dependency were convincing to attribute them unequivocally to the treatment with metam-sodium.

Checking the findings with the criteria in the CLP guidance:
«Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.
Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.».

When assessing the criteria in points 3.7.2.4.1 through 3.7.2.4.3, RMS considers that the findings are concerning enough to classify metam-sodium as a suspected developmental toxicant, albeit recognising that the foetotoxic effects are probably not specific, given the consistent presence of overt maternal toxicity, at least when considered throughout the outcome of the 7 developmental studies in both the rat and the rabbit. In cases where dose-dependency was noted, the effects are minor, like those associated with retardations of ossifications. The occurrence of more severe malformations (notably meningiocoele) is characterised by the absence of a convincing dose-dependent response and/or a low incidence.

Overall, RMS would therefore propose to classify metam sodium as a CATEGORY 2, Suspected human reproductive toxicant (Repr.2, H361d «Suspected of damaging the unborn child»).

Table 61a: Metam sodium, overview of the rat developmental studies

		dose (mg/kg bw/d)						
Reference:		**5**	**10**	**20**	**40**	**60**	**80**	**120**
[redacted], 1987a **(B.6.6.2.1 /01)**	MT		[↓bw(g)]		**↓bw(g)**			**↓bw(g)**
			[↓fc]		**↓fc**			**↓fc**
	FT							↓bw
					↑resorption (#1)			↑resorption (#2)
								↑PIL
								meningocoel (#1)
								μphtalmia (#1)
			↑var		↑var			↑var (?)
[redacted], 1993a (RF) **B.6.6.2.1 /02**	MT			↓bw(g)	**↓bw(g)**		**↓bw(g)**	
				↓fc	**↓fc**		**↓fc**	
							↑stomach erosion	
	FT			↓bw	↓bw		↓bw	
							meningocoel (#1)	
[redacted] 1993b **B.6.6.2.1 /03**	MT	[↑clin. sgn.]		↑clin. sgn.		**↑clin. sgn.**		
		[↓bw]		↓bw		**↓bw**		
						↑kidn. pelvic dil.		
	FT			↓bw		↓bw		
						↑PIL		
						↑uterine death		
						↑hydrocephalus (#3)		
						μphtalmia (#2)		
		↑min/var		↑min/var		↑min/var		

(RF): range-finding study; MT: maternal toxicity (in **bold** in case of serious foetal effects at the same dose); FT: foetal toxicity/developmental effects; [..]: borderline/insignificant effect, but trend-like; (?): uncertain, as no dose-effect; (#): number of foetuses affected; ↑↓: toxicological/significant changes as compared to controls (not tabulated)
Bw(g): body weight (gain); clin. sgn: clinical signs; fc: food consumption; min/var: minor defects/variations; PIL: post-implantation loss, μphtalmia: microphtalmia; kidn. pelvic dil.: kidney pelvic dilatation

Table 61b: Metam sodium, overview of the rabbit developmental studies

		dose (mg/kg bw/d)							
Reference:		**5**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
[redacted], 1987b **B.6.6.2.2/ 04**	MT				**↓bwg**				**↓bwg**
	FT		↑PIL		↑PIL				↑PIL
			↑uterine death		↑uterine death				↑uterine death
					gall bladder agenesis (#1)				gall bladder agenesis (#1)
			↑var		↑var				↑var
[redacted], 1993a (RF) **B.6.6.2.2/01**	MT							↓bw(g)	
								↑clin. sgn.	
[redacted] 1993b (RF) **B.6.6.2.2/ 02**	MT			↓bwg		**↓bwg**	**↓bwg**		
		[↓fc]		↓fc		**↓fc**	**↓fc**		
		[↑clin. sgn.]		↑clin. sgn.		**↑clin. sgn.**	**↑clin. sgn.**		
							↑stomach erosion		
	FT					↓bw	↓bw		
						↑PIL	↑PIL		
						↑uterine death	↑uterine death		
						↓live foetuses	↓live foetuses		
							cyclopia (#1)		
[redacted] 1993c **B.6.6.2.2/ 03**	MT	[↓bw(g)]		↓bw(g)			**↓bw(g)**		
				↑clin. sgn.			**↑clin. sgn.**		
	FT						↓live foetuses		
							↑PIL		
							↑uterine death		
							meningocoel (#1)		
		cleft palate (#1)					↑cleft palate (#2)		
				↑min/var			↑min/var		

(RF): range-finding study; MT: maternal toxicity (in **bold** in case of serious foetal effects at the same dose); FT: foetal toxicity/developmental effects; [..]: borderline/insignificant effect, but trend-like; (?): uncertain, as no dose-effect; (#): number of foetuses affected; ↑↓: toxicological/significant changes as compared to controls (not tabulated)
Bw(g): body weight (gain); clin. sgn: clinical signs; fc: food consumption; min/var: minor defects/variations; PIL: post-implantation loss, μphtalmia: microphtalmia
(red highlight: serious embryonic/foetal adverse effect at a dose <MT, in 1/3 developmental studies)

MITC

As discussed hereabove (**2.6.6.2.1**), RMS considers that MITC is devoid of reprotoxicity hazard, and the slight effects observed at maternotoxic doses are insufficient to propose a classification for reprotoxic toxicity under the CLP regulation.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 62: Summary table of animal studies on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 63: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

Table 64: Summary table of other studies relevant for effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Metam and MITC: no data available				

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

Multigeneration studies have been submitted for both metam-sodium and its main metabolite MITC.

Metam

In the 2-generation study with Metam, the a.s. was administered to groups of 30 ♂ and 30 ♀ Wistar-derived rat strain (F0 parental generation) as a constant addition to the drinking water at nominal concentrations of 0, 0.01, 0.03 and 0.10 mg/mL, corresponding with 1.4, 4.0, 12.3 mg/kg body weight/day (♂) and 2.0, 4.9, 14.4 (♀). The outcome and conclusion of this 2-generation study conducted with **metam** was as follows

Nasal passage of the majority of F_0 and F_1 adult ♀ and more specifically Bowman's (olfactory) gland was hypertrophied at the top-dose. There was no effect on the nasal passages of adult ♂ or offspring. This suggests that the nasal lesion was a direct consequence of the increased dose received by ♀ during lactation.

Water consumption was ↓at top dose and was accompanied by slight ↓in body weight, which were most evident during gestation and lactation in adults.

Effects in pups included a slight ↓in individual pup weight and in total litter weight in the top-dose group in both generations. There was no indication of a change in the nasal passage of offspring of either generation at any dose level.

The NOAEL parental toxicity= 4.0 mg/kg b.w./d, based on the ↓body weight during premating, gestation and lactation reported at 12.3 mg/kg b.w./d.

The NOAEL offspring= 4.0 mg/kg b.w./d, based on the ↓pup and litter weight observed at 12.3 mg/kg b.w./d

The NOAEL reproduction toxicity = 12.3 mg/kg b.w./d, the top-dose.

RMS observes that no study was submitted investigating the transfer of a.s. or any of its metabolites in the mother milk (neither in the rodent ADME studies nor in farm animals).

No adverse findings have been identified which would indicate a transfer of test article or its degradates in the maternal milk causing adversity in the offspring offspring via this way.

Considering the partition coefficient of metam (<-2.9), the likelihood of transfer to the mother milk and consecutive exposure of the offspring via this way is low.

Therefore, RMS considers that no classification is needed and no further data should be required either.

MITC

RMS observes that no study was submitted investigating the transfer of a.s. or any of its metabolites in the mother milk (neither in the rodent ADME studies nor in farm animals).

In the 2-generation study with **MITC**, SD rats were exposed to MITC by inhalation at 0, 1, 5, and 20 ppm (0, 3, 15, and 60 mg/m³), corresponding to about 0.8, 4.05 and 16 mg/kg b.w./d).

In the 2-generation study conducted with MITC via inhalation, F_1 pups (**litters**) that were found dead or euthanised *in extremis* from PND 0 through the selection of the F_1 generation on PND 28 were 16(**11**), 23(**8**), 27(**7**), and 22(**11**),

whilst F_2 pups (**litters**) that were found dead or euthanised *in extremis* from PND 0 through the selection of the F_2 generation were 28(**15**), 11(**7**), 15(**7**), and 6(**5**) in the control, 1, 5, and 20 ppm groups, respectively. It is of note that in this generational study with MITC, the parental (systemic) and offspring NOAEL was set at 5.0 ppm and 20 ppm, respectively, while the reprotoxicity NOAEL was set at 5 ppm, on the basis of ↑oestrous cycle length (F_0,F_1); and gestation length (F_0,F_1), and delayed vaginal patency (F_1).
It was concluded that the mortality, and hence, occasional findings of milk absence in the pup stomach were not dose-related, and therefore not substance-related.

Considering the partition coefficient of MITC (1.05) the likelihood of transfer to the mother milk and consecutive exposure of the offspring via this way is low. MITC is highly volatile (vapour pressure at 20°C: 1739 Pa) and readily soluble in water (8.94 g/L at 20°C) and moderately volatile from water (H = 14.2 Pa.m^3/mol at 20 ºC). With a moderate log P_{ow} of 1.05, bioaccumulation of MITC is not expected.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

Regarding the criteria in the CLP guidance (3.7.2.2.2) for substances causing effects on or via lactation, no human data are available.
Classification for effects on or via lactation can be assigned on the basis of toxicokinetic data (which are present, but harbouring no specific measures of test articles in the milk), or a well substantiated estimate of the exposure through the milk alone (in generational studies) provided that it is supported by an argument clearly justifying that the level present in the breast milk would be likely to harm developing offspring.

Metam
In the summary described above, is not considered that fertility or pup growth and development was specifically affected in the 2-generation study of metam administered via the drinking water. There was however also no estimate of the exposure through the milk alone, taking into account that there was no particular reason on the basis of physical-chemical properties of metam (log P_{ow} <-2.9) indicating no lipophilicity or any bio-accumulating property. Therefore, there is no suspicion that the substance would be prone to migrate to the maternal milk, and harming the offspring via that way, and further data were not generated.

Therefore, RMS considers that no classification is needed for metam regarding effects on or via lactation, and no further data should be required either.

MITC
In the summary described above (details to be found in Vol.3 B.68.1.5.1/01 thru 03), is not considered that fertility or pup growth and development was specifically affected in the 2-generation study of MITC administered via inhalation.
The observed mortality in pups were equally dispersed among the different treatment groups, including controls, without dose-response. Hence, mortality, and occasional observation of milk shortage in pup stomach, was not attributed to treatment. The presence or absence of milk in the stomach the latter is an indication of the incapability of nursing pups, but without association with MITC treatment. To demonstrate that MITC would cause adverse effects on or via lactation it should be evident that there would be ↑ pup mortality during lactation, particularly between PND 4 and PND 21 at least in the F2 generation, where enough time would be elapsed to allow accumulation of the test article in mammary gland and/or maternal blood. In the absence of such findings, there is no indication in generational study on rats, that MITC would interfere via lactation on pup development.
There was however also no estimate of the exposure through the milk alone, taking into account that there was no particular reason on the basis of physical-chemical properties of MITC (log P_{ow} 1.05) indicating no strong lipophilicity or bio-accumulating property. Therefore, there is no suspicion that the substance would be prone to migrate to the maternal milk, and harming the offspring via that way, and further data were not generated.

Therefore, RMS considers that no classification is needed for MITC regarding effects on or via lactation, and no further data should be required either.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Metam
Classified - Repr.2, H361d «Suspected of damaging the unborn child»

MITC
Not classified - not sufficient for classification

2.6.7 Summary of neurotoxicity

Table 65: Summary table of animal studies on neurotoxicity

Table 65a: Summary of neurotoxicity of Metam.

Type of test; test species; tested doses and actual intake; Guideline, GLP compliance, acceptable or not	Test substance , Batch n°, purity	Results			References
		NOAEL	LOAEL	Observed effects	
Oral acute (gavage) study, rat, (Crl:CD® BR) 0, °50, 750, °1500 mg/kg bw/d (12-16/sex/dose) No guideline followed GLP compliant, supplemental	Metam sodium B. n°: not reported Stated purity: 43.15%	Systemic: 50 mg/kg bw Neurotoxic: <50 mg/kg bw	Systemic: 750 mg/kg bw Neurotoxic: 50 mg/kg b.w.	↓body weight, ↓water consumption, slight ↓food consumption (younger ♀) *Top-dose:* ↑mortality. ↓(loco)motor activity *≥750 mg/kg bw* alterations in posture and palpebral closure; lachrimation, salivation, fur appearance, respiratory rate); alterations in arousal and gait, ↑time to 1st step, ↓rearing activity); absent approach, olfactory and pupil responses, absent tail pinch response, ↓tail pinch stimulus reaction time, ↓startle response; ↓hindlimb extensor strength).	[redacted], 1993a - °b **B.6.7.1/01** **°B.6.7.1/02**
Oral 90-day drinking water study, rat (Alpk:APf SD-Wistar derived) 0, 0.02, 0.06 and 0.20 mg/mL (12/sex/dose) Actual intake (mg/kg bw/d) ° 0, 2.0, 6.0, 14.7 (♂) 0, 3.3, 8.4, 17.8 (♀) Comparable to OECD 424 (1997) GLP compliant, acceptable	Metam sodium B. n° BAS/005/00N Purity: 525.54 g/L	Systemic: <2 mg/kg bw/d Neurotoxic: 14.7 mg/kg bw/d	Systemic: 2 mg/kg bw/d Neurotoxic: >14.7 mg/kg bw/d	↓body weight ↓food consumption No signs of neurotoxicity.	[redacted], 1994 **B.6.7.1/03**

°: Doses in the companion acetylcholinesterase assay (**B.6.7.1/02**, considered supplemental) limited to control and top-dose animals

Table 65b: Summary of the acute neurotoxicity of MITC

Type of test; test species, test substance MITC – batch number – purity; tested doses; Guideline, GLP	Results			References, Study n°
	NOAEL	LOAEL	Findings	
Inhalation acute neurotoxicity study, SD- rat **MITC,** B.n°: 51198PJV; 99.7%	Local <20 ppm <60 mg/m³ **<16 mg/kg bw/d**	Local: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	↑olfactory epithelium necrosis, ↑transitional epithelium degeneration on d1.	[redacted], 2011b, **B.6.8.1.6/01**

Type of test; test species, test substance MITC – batch number – purity; tested doses; Guideline, GLP	Results			References, Study n°
	NOAEL	LOAEL	Findings	
Dose levels : 0, 20, 40, 80 ppm 0, 60, 120, 240 mg/m³ 0, 16, 32, 65 mg/kg bw/d OECD 424 (1997) GLP compliant The selected doses are acceptable, with the top-dose (240 mg/m³) being ~40% LC_{50} (540 mg/m³).	Systemic: <20 ppm <60 mg/m³ **<16 mg/kg bw/d**	Systemic: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	↓absolute and relative liver weight *At ≥40 ppm:* ↑clinical signs; *At 80 ppm:* ↓b.w. (change).	
	Neurotoxic: 40 ppm 120 mg/m³ **32 mg/kg bw/d**	Neurotoxic: 80 ppm 240 mg/m³ **65 mg/kg bw/d**	↑axonal degeneration, possibly associated with hindlimb functionality (d15). Note: NT observations confounded by systemic toxicity: *At ≥20 ppm:* ↓rotarod performance, ↑eyelids completely and/or half-closed; *At ≥40 ppm:* ↑slightly soiled fur appearance, ↑oral crusty deposits, ↓rearing , ↑grooming activity; ↓locomotor activity (d0), unremarkable or less prominent at d7 and totally resorbed at d14.	

Short summary and overall relevance of the provided information on neurotoxic effects

Metam sodium

An acute neurotoxicity study was performed with metam sodium administered to rats at 50, 750 or 1500 mg/kg bw by gavage ([redacted], 1993a, .**B.6.7.1/01**). Based on ↓bodyweight (gain) and ↓body temperature at 750 mg/kg b.w onwards, it appears that the NOAEL for systemic toxicity may be set at the lowest dose. Mortality occurred at the top-dose of 1500 mg/kg b.w..
Most potentially acute neurotoxic observations were also observed at 750 mg/kg b.w onwards. These effects included adverse effects in home cage and handling observations (alterations in posture and palpebral closure; lachrimation, salivation, fur appearance, respiratory rate), open field observations (alterations in arousal and gait, ↑time to 1st step, ↓rearing activity), sensimotor observations (absent approach, olfactory and pupil responses, absent tail pinch response, ↓tail pinch stimulus reaction time, ↓startle response), and neuromuscular parameters (↓hindlimb extensor strength).
However, in open-field observation, both total motor and ambulatory activity were decreased from the lowest dose onwards. The position of the notifier, that the adverse effects observed at all dose levels were attributed to systemic toxicity and /or local gastrointestinal irritation of metam sodium is not supported, since even at acutely toxic doses, these effects are not necessarily observed in all cases.
It may thus be discussed whether changes in (loco)motor activity (affected at all doses, including 50 mg/kg bw) may reflect a specific effect of metam on the nervous system, but according to the RMS, it is observed at a dose below that causing overt systemic toxicity should be considered *potentially* neurotoxic and the NOAEL established accordingly. However, **RMS** recognises that this study is conducted at high dose levels, and that it is difficult to differentiate between direct and indirect effects on the nervous system.
NOAEL systemic toxicity = 50 mg/kg bw.
NOAEL acute neurotoxicity <50 mg/kg bw.

In an additional study ([redacted], 1993b, **B.6.7.1/02**) the effect of metam sodium on red blood cell, plasma and brain cholinesterase activity was determined after a single oral dose (gavage) of 1500 mg/kg bw to Sprague-Dawley rats. No effects on plasma, red blood cell or brain cholinesterase levels were observed at dose level of 1500 mg/kg bw at 45 minutes (study day 0) or 24 hours (study day 1) post-dosing.
No effects were observed in the 1500 mg/kg bw group on absolute brain and brain region weights, brain and brain region weights relative to final body weights or brain region weights relative to brain weights at 45' (day 0) or 24

hours (day 1) post-dosing. In conclusion, neurotoxicity of metam sodium was not associated with blood/plasma acetylcholinesterase inhibition. It is noted that brain acetylcholinesterase inhibition was not assessed.

In a subchronic neurotoxicity study ([redacted], 1994, **B.6.7.1/03**), metam sodium was administered during 90 to rats via the drinking water.
Under these experimental conditions, metam sodium at dose levels up to 0.2 mg/mL, equivalent to 14.7 mg/kg b.w./d, does not show signs of subchronic neurotoxicity.
A slight significant decrease in both body weight and food consumption was observed at the lowest dose onwards.
Therefore, the **systemic toxicity NOAEL** was **< 2.0 mg/kg b.w./d**. In the regular OECD-compliant 90d study in rats of the same strain a NOAEL was identified at 0.5 mg/kg b.w./d.
The **subchronic neurotoxicity NOAEL = 14.7 mg/kg bw/d**.

No study on delayed polyneuropathy was performed with metam. As metam is not an organophosphate, did not induce any neurotoxic effects and did not inhibit plasma, erythrocyte or brain cholinesterase, a study investigating delayed polyneuropathy is not required.

In the open scientific literature, 2 articles suggesting a possible DNT effect in mice at <50 mg/kg b.w./d. [redacted], 2020, **B.6.8.2.4/03, and 2021, B.6.8.2.4/04**). Although these published study are considered supplementary information only, the question could be asked whether the absence of a guideline DNT would constitute a data gap.

Conclusion:
Since the acute neurotoxicity assays are at the limit of acceptability, the results are considered to provide complementary information only. Since a valid 90-d study exists, **RMS** considers the neurotoxicity endpoints sufficiently covered, in the absence of neurotoxicity findings in the latter study.
Despite the fact that metam belongs to the class of dithiocarbamates, and the likelihood that minor amounts of CS_2 can be released, RMS considers that, throughout the existing toxicological database, the toxicity of metam-sodium is not driven by neurotoxic adverse effects. Taking into account the reference dose of MITC, used in the human health risk assessment, the Margin of Safety is 14.7÷0.004 = more than a factor of 3000.

MITC

In a combined acute inhalation toxicity/neurotoxicity study ([redacted], 2011b, **B.6.8.1.6/01**), SD-rats were exposed to a single, 6-hour whole-body inhalation at target exposure concentrations of 20, 40, and 80 ppm (equivalent to 60, 120 and 240 mg/m³), corresponding to mean measured exposure concentration of 20, 42 and 82 ppm (equivalent to 60, 126 and 246 mg/m³). The study was divided into 2 phases, *i.e.* a Satellite Phase in order to characterise the toxicological response following single high-level exposures and a Core Phase for the evaluation of the neurotoxicity potential of the test substance following exposure towards identical exposure levels as employed in the Satellite Phase. For pathological examinations and for the assessment of reversibility of effects after a single 6-hour exposure, the Core Phase animals were split into a neuropathology and a toxicity subgroup.

A **local NOAEL** was set to **< 20 ppm**, based upon olfactory epithelium necrosis and transitional epithelium degeneration (satellite phase) and olfactory epithelium degeneration (core phase) in the nose from 20 ppm onwards. In the bronchoalveolar lavage (BAL), a decreased number of macrophages and an increased number of neutrophils were found at the top-dose.

A **systemic NOAEL** was also set to **< 20 ppm**, based on a dose-dependent decrease (>10%) in both absolute and relative liver weight at 20 ppm onwards (corresponding to **<60 mg/m³** or **<16 mg/kg b.w./d**), attaining statistical significance at 40 ppm and above. Clinical signs (d1) were observed at 40 ppm and above in both satellite and core phase.
Body weights were decreased at the top-dose in both satellite and core phase, b.w. changes were decreased at ≥20 ppm, but only in the satellite phase (d0-1 period).

Regarding specific neurotoxic effects, the study results indicated decreased (>10%) rotarod performance and hindlimb footsplay, and a FOB cage-side observation (eyelids completely and/or half-closed) at the lowest dose of 20 ppm onwards. At 40 ppm and above, FOB handling observation revealed slightly soiled fur appearance and oral crusty deposits, and decreased rearing and increased grooming activity were observed during the open-field observations. At these dose-levels, locomotor activity was also decreased.
However, the abovementioned neurotoxic effects, observed on d0 were unremarkable or less prominent at the mid-point (d7), and totally resorbed at the end of the neurotoxicity study (d14).
At the top-dose though, a few specific nerves showed axonal degeneration, possibly associated with hindlimb functionality at study termination (d15).

Whereas no dose-level without effect could strictly be identified, for local, systemic and neurotoxicity (although the latter is characterised by a complete resorption of the neurotoxic signs on d14 in this study), the overall assessment points to a neurotoxicity profile which cannot be dissociated from the observed upper airway corrosive and general (systemic) toxicity of MITC. However, it cannot completely be excluded that the isolated cases of axonal degeneration could in some way be associated with more specific neurotoxicity at the top-dose-level op 80 ppm.

Therefore, an acute **neurotoxicity NOAEL** could be cautiously set at **40 ppm,** corresponding to **120 mg/m³** or **32 mg/kg b.w./d**, based on axonal degeneration at **80 ppm**, corresponding to **240 mg/m³** or **65 mg/kg b.w./d**.
It should be acknowledged that this potential neurotoxicity was observed at an acute dose corresponding with about 40% of the LC_{50} of MITC (about 540 mg/m³), and that this endpoint could be confounded by the high systemic toxicity caused by MITC.

The overall conclusion is that MITC does not present a *specific* acute neurotoxic hazard.

Consequently, **RMS** is of the opinion that there is no need for the conduct of a subacute or subchronic neurotoxicity study, in the reasonable assumption that the existing dataset of toxicity studies with MITC after repeated administration is informative enough on more critical toxicity effects, covering any potential neurotoxic endpoint.

2.6.8 Summary of other toxicological studies

1. Toxicity studies of minor metabolites and relevant impurities

Minor volatile metabolites, other than the main metabolite MITC, include: methylisocyanate (MIC), methylamine, hydrogen sulphide (H_2S), carbonyl sulfide (COS), and carbon disulfide (CS_2). DMTU, as a potential impurity, metabolite or degradation product, plausibly formed as a reaction between MITC and methylamine, is also discussed.
All these metabolites are toxicologically relevant, but may not be considering increasing the overall risk in the light of their occurrence, and/or of the more critical toxicological profile of metam and MITC. As they are also formed in the mammals, and as the toxicity of both metam and MITC may conceivably be the result of synergy (and possibly antagonism) of all substances formed, the extensive toxicological package is considered to cover all hazards. As a more general guidance, the comparison of the harmonised or proposed classifications under CLP is to a certain extend also indicative, as none of them show classifications for human health which would rise a particular extra concern, when compared to metam and MITC.

Data were mostly extracted from published literature sources; a summary of the inhalation NOAEL's of these metabolites is given below:

Metabolite	**ppm and mg/m³**	**NOAEL**
Methylisocyanate	1 ppm = 2.55 mg/m³	1.53 mg/m³
MITC	1 ppm = 3.26 mg/m³	5 mg/m³
Methylamine	1 ppm= 1.27 mg/m³	95 mg/m³
H_2S	1 ppm = 1.52 mg/m³	15.2 mg/m³
COS	1 ppm = 2.6 mg/m³	780 mg/m³
CS_2	1 ppm = 3.3 mg/m³	10 g/m³ (LC_{50})

Methyl isocyanate (MIC)
Based on the hazard profile available for MIC, this metabolite does not increase the hazard compared to MITC when released during chemical reaction/degradation of MITC. Both are considered acutely toxic after inhalation currently resulting in the same classification. However, the classification for MIC for acute inhalation is currently a minimum classification. MITC is considered corrosive and therefore classification is stricter than for MIC covering skin/eye irritation and consequently respiratory effects. MITC is also considered a skin sensitiser. The effects on development by MIC are not considered relevant for hazard assessment for MITC, as it is considered secondary to general toxicity. In addition to the comparable hazard profile of MITC and MIC, based on the low real concentrations of MIC as mentioned in the field trial of [redacted] (2006, see product DAR), no increase of risk is estimated in case of release of MIC from MITC.

Hydrogen sulfide (H_2S)
Based on the hazard profile available for H_2S, this metabolite does not increase the hazard compared to MITC when released during degradation of metam to MITC. Both are considered acutely toxic after inhalation currently resulting

in the same classification. No other harmonised classification was deduced to H_2S. The effects related to neurotoxicity were observed only at high dose levels in the range of LC_{50} values and are thus considered not critical for the assessment but covered by the acute toxicity. In addition to the hazard profiles of MITC and H_2S, based on the low measured concentrations of H_2S (8-76 ppb up to 24 h after metam application) no increase of risk is assumed in case of release of H_2S from MITC.

Carbonyl sulfide (COS)

Based on the hazard profile available for COS, this metabolite does not increase the hazard compared to MITC when released during cleavage of MITC in the gut. Both are considered acutely toxic after inhalation. However, COS is classified in category 3 (notified classification) whereas MITC is classified in category 2. Other effects deduced to COS included irritating (local) effects. The effects related to neurotoxicity were observed at 400-600 ppm in rats. NOAEL values for systemic and neurotoxic effects were in the range of 200-300 ppm in subchronic studies. Similar results were observed in subacute studies indicating that the exposure duration is less critical. In one study in Sprague-Dawley rats exposure for 17 weeks by inhalation, resulted in a NOAEL of 60 ppm and a LOAEL of 182 ppm for reprotoxicity endpoints (decreased pregnancy rate). In rabbits a NOAEL (for cardio and respiratory effects) and LOAEL of 54 ppm (systemic mortality) were observed in studies with 7 weeks exposure. In addition to the hazard profiles of MITC and COS, and the fact that COS is considered to be produced in the gut, the exposure to COS is considered low and therefore no increase of risk is estimated in case of release of COS from MITC.

Methylamine (CH_3NH_2 or MMA)

Based on the hazard profile available for MMA, this metabolite does not increase the hazard compared to metam when released upon cleavage of metam sodium under acidic conditions. Both are considered acutely toxic after inhalation and corrosive. Similar to H_2S the effects related to neurotoxicity of MMA were observed only at high dose levels in the presence of local and/or general systemic effects. In addition to the hazard profiles of metam and MMA, based on the fact that MMA (LOD: 27 µg/m³) was not detected around the treated field, no increase of risk is estimated in case of release of MMA from metam.

Carbon disulfide (CS_2)

Based on the hazard profile available for CS_2, this metabolite does not increase the hazard compared to metam when released upon cleavage of metam sodium under acidic conditions, except for the effects observed for reproductive toxicity/neurotoxicity. In addition to the hazard profiles of metam and CS_2, based on the fact that CS_2 (LOD: 4 ppb or 13 µg/m³) was not detected around the treated field, no increase of risk is estimated in case of release of CS_2 from metam.

Dimethylthiourea (DMTU)

Based on the hazard profile available for DMTU, this metabolite does not increase the hazard compared to MITC when released during chemical reaction/degradation of MITC. DMTU is not acutely toxic after oral intake. No harmonised classification exists for DMTU, and few studies are available. The potential effects on development by DMTU (as is known for many thiourea in general) are not considered critical, since findings occur at a dose of 500 mg/kg bw/d, which is 100× higher than the lowest developmental rat LOAEL. In addition to the comparable hazard profile of DMTU and metam, and considering low levels of DMTU in the TGAI (around 1%) no increase of risk is estimated in case of exposure to DMTU under the current GAP. Likewise, in a read-across, DMTU may display carcinogenic effects (thyroid tumours) at high doses in the rat. Considering a carcinogenicity LOAEL of 25 mg/kg bw/d for DMTU, this compares favourably against the carcinogenicity of metam, which is around 2 mg/kg bw/d, and overall, taking into account the low level of DMTU in the TGAI, is unlikely to impact the risk assessment of the a.s. itself.

In a worse-case approach, its toxicity was considered equivalent to the parent compound Metam, which is also proposed to be classified as a Cat 2 carcinogen and developmental toxicant. While the a.s., but not MITC is possibly a candidate for a classification as a Cat 2 genotoxicant, it appears that a reliable QSAR run on DMTU also provides an equivocal result, and in the absence of genotoxicological studies could be considered equivalent to metam itself, implying no additional risk from genotoxicological point of view.

Based on these informations, RMS considered DMTU as being potentially a toxicologically relevant compound. In addition, since metam-sodium was tested for mammalian toxicity with batches containing 1% of DMTU, it may be inferred that all toxicity endpoints and related classifications sufficiently covered the toxicity of this impurity in the technical materials.

It may be concluded that the effects of DMTU are covered by the toxicological and ecotoxicological data package; there is no residue situation, and as regards its toxicological profile, it should not be included in the residue definition for risk assessment.

General conclusion

As a general conclusion, RMS is of the opinion that both hazard and risk assessment of the a.s. is driven by metam itself and its main metabolite MITC. Although further minor breakdown products of toxicological relevance may

be formed and/or are present as impurities, they are probably also metabolites which can be formed in mammals in a certain degree, and are thus likely covered by the vast toxicological package. In addition, considering their low presence in the environment following application under the current GAP, and their absence in residues, it is concluded that they are unlikely to increase the toxicological consumer and non-consumer risk of metam and MITC.

2. Immunotoxicity studies on metam and MITC

MITC

In a 28-day whole-body inhalation immunotoxicity study ([redacted] 2011b, **B.6.8.1.7/01**), ♀$B_6C_3F_1$ mice were exposed to MITC vapour at target concentrations of 0, 1, 3 and 10 ppm (actual concentrations: 0, 1.05, 3.09 and 10.04 ppm, equivalent to 0, 3.15, 9.27 and 30.12 mg/m³) for 28 consecutive days, 6 hours per day and 5 days a week.

No suppression of the humoral component of the immune system was observed, at a top-dose which was however at the limit of adverse systemic toxicity, since body weight was only marginally affected.
In the view of minimal systemic toxicity achieved at the top-dose, and the limited number of immunological parameters investigated (only spleen and thymus weight, and AFC assay), this study is considered to provide complementary information, only. Both **systemic NOAEL** and **immunotoxicity NOAEL** were **> 10 ppm** (**30 mg/m³**, or **13 mg/kg bw/d**), the highest exposure concentration tested.
Neither spleen weight nor thymus weight were affected at any dose level, and there was also no treatment-related effects of MITC in the antibody-forming cell (AFC) assay, indicating no effect on the humoral immune response.

While some published studies (*e.g.* Keil et al, 1996) suggested a specific immunologic modulation by MITC at relatively high doses (45 mg/kg bw/d in a 5d- oral study on ♀$B_6C_3F_1$ mice), a confounder with systemic toxicity is possible. In another pilot 28d- inhalation study on ♀$B_6C_3F_1$ mice ([redacted], 20013), meaningful drops of thymus weight were noted at doses at or above the MTD, *i.e.* 16, 32 and 64 mg/kg bw/d. Overall, these effects on the thymus are observed at systemically adverse dose-levels; suggesting that it could be associated to general toxicity or associated stress. However, it should be acknowledged that the existing test procedures are limited to organ weights and limited functional tests, and in the absence of validated OECD immunotoxicity assays in mice or other susceptible species, the issue remains a data gap for a.s. evaluations in general.

In conclusion, while intrinsic immunological effects cannot be excluded entirely, it is considered that the critical effects of MITC are shown at much lower doses, and derived reference values from these are covering any potential direct or indirect immunological effects. It is also highlighted that throughout the toxicological database, no signs were noted which would raise suspicion for a compromised immunological status (*e.g.* opportunistic infections) of tested animals in any study, including those covering long-term exposures, neither via oral nor via inhalatory routes of entry.

Summary of the guideline MITC immunotoxicity study

Type of test; test species, tested doses; Guideline, GLP	**test substance batch number, purity**	**Results**			**Reference study n°**
		NOAEL	**LOAEL**	**Findings**	
28-Day inhalation immunotoxicity study, **♀mouse ($B_6C_3F_1$)** Dose levels : 0, 1, 3, 10 ppm 0, 3, 9, 30 mg/m³ 0, 1.4, 4, 13 mg/kg bw/d OECD 424 (1997) GLP compliant RMS : in the view of minimal toxicity achieved at the top-dose, and the limited number of immunological parameters investigated, this study is considered to provide **complementary information, only**.	**MITC** Batch n° 51198PJV, Purity 99.7%	Systemic: 10 ppm 30 mg/m³ **13 mg/kg bw/d**	Systemic: >10 ppm >30 mg/m³ **>13 mg/kg bw/d**	No adverse effect	[redacted] (2011b) **B.6.8.1.7/01**
		Immunotoxicity: 10 ppm 30 mg/m³ **13 mg/kg bw/d**	Immunotoxicity: >10 ppm >30 mg/m³ **>13 mg/kg bw/d**	No adverse effect	

Metam

It is of note that no guideline immunotoxicity study was conducted on the a.s. metam itself.

However, in the open scientific literature, a number of publications were retrieved which treated the effect of metam-sodium and its degradation product on immune-sensitive organs and tissues. In a number of them, specific endpoints were investigated which could be useful to understand the genetic background and concomitant expression of a.o. Toll-like receptors (TLRs) playing crucial roles in the innate immune system by recognising pathogen-associated molecular patterns derived from various micro-organisms. TLRs signal through the recruitment of specific adaptor molecules, leading to activation of the transcription factors like NF-κB and IRFs, which dictate the outcome of innate immune responses. All studies emerged from a NIEHS-funded research programme, hosted by the team of Stephen Pruett of the Mississippi State University,

In an initial publication (Pruett *et al*, 1992), the immunotoxicity after *in-vivo* oral (gavage) administration of Metam-Na at the dose of 300 mg/kg b.w./d for 3, 5, 10 or 14 days to 6-10 wk old ♀$B_6C_3F_1$ mice was investigated. At this dose, a decrease of thymus weight (–70% abs., –68% rel. to b.w.) and an increase of spleen weight (+36% abs, +50% rel.) was observed, along with a significant decrease of b.w. gain on d14. Significant changes in mature lymphocyte subpopulations in the blood (differential ↓lymphocytes and ↑neutrophils, in the presence of a slight ↑WBC count), along with ↓RBC parameters were observed, while the bone marrow cellularity was high (+37%). The major subpopulation of thymocytes ($CD4^+CD8^+$, *i.e.* immature T cells) was depleted selectively in the thymus. In the thymus and the spleen, the fraction of $CD4^-CD8^-$ was increased (in the spleen, the latter 'non-B, non-T' subpopulation represents phagocytes and haemopoietic cells, the increase of which could be illustrative of a compensatory reaction).
In addition, administration of Metam-Na (200 mg/kg b.w./d orally for 7d, or 200-300 mg/kg b.w./d dermally for 4d) caused a substantial, dose-dependent suppression of splenic 'natural killer' (NK) cell activity (apparently in the absence of b.w. effects, thus at doses which are not excessively toxic). No suppression of IgM-antibody production *in vivo* or splenocyte responses to B- or T-mitogens or allogeneic lymphocytes *in vitro* was detected. While *in-vitro* humoral immune functionality was affected *in-vitro*, such an effect was not observed *in-vivo.*
In conclusion, the authors suggested that, while humoral immune response was not a major target for acute effects of Metam-Na, NK cell function (*i.e.* cellular immunity) was affected.

In Keil *et al*. (1996), the major decomposition product of Metam-Na, methylisothiocyanate (MITC), and two minor products, methylamine and carbon disulfide, were investigated for their potentiel immunotoxicological capacity in the $B_6C_3F_1$ mouse. Metam-Na (300 mg/kg b.w./d), carbon disulfide (137 or 551 mg/kg b.w./d), or MITC (15, 30 or 45 mg/kg b.w./d) were administered by gavage to 8-10 wk old ♀$B_6C_3F_1$ mice (n=5) for 5-7d, and methylamine (122, 488 or 977 mg/kg b.w./d) was administered *i.p.*. Terminal b.w./spleen/thymus weight, WBC counts and differentials, thymocyte subpopulation quantification and NK cell activity (lysis of ^{51}Cr-labelled YAC-1 tumour cells by splenocytes *in-vitro*) were investigated.
The most prominent effects of metam included, again, a decrease in both thymus weight and relative amount of $CD4^+CD8^+$ thymocytes, an increase in spleen weight, and altered percentages of neutrophils (↑) and lymphocytes (↓) in the blood. In addition, a decrease of the natural killer (NK) activity was noted in splenocytes.
It was observed that equimolar (to Metam-Na) doses of methylamine and CS_2 caused minimal immunological changes (notably no decrease of both thymus and spleen weight, although Keil et al highlighted an observed meaningful decrease of total WBC, which, in the absence of modifications in immune-sensitive organ weight and of WBC subpopulations, cannot be interpreted as an immunotoxic effect *per se*) , and these changes were not characteristic of those noted for Metam-Na.
In contrast, MITC at 45 mg/kg b.w./d for 5-7d, also significantly decreased the thymus (but not the spleen) weight (about –50%). A shift in the peripheral white blood cell populations (↑neutrophils, ↓lymphocytes, total WBC not significantly altered) was observed, but it was of note that no dose-dependency existed (significant change at 15, but no change at 30 mg/kg b.w./d). Alterations of the thymocyte populations (↑$CD4^+CD8^+$ and ↓$CD4^-CD8^-$, as in metam) was noted at the top-dose dose. MITC did not significantly affect NK cell activity (as metam did at 300 mg/kg b.w./d) or increase spleen weight. It was postulated that the parent compound, or a synergistic action of the parent compound with one or more decomposition products, could be responsible for the remaining changes (increased spleen weight and decreased splenic NK cell activity).
It was remarked that in this study, consistent immunological effects of MITC were only observed at a high dose (45 mg/kg b.w./d). In this study, 55 mg/kg b.w./d was considered near-MTD, and the oral LD_{50} of MITC in the ♀ mouse was about 100 mg/kg b.w.. Therefore, the involvement of systemic toxicity was suspected (although the authors reported a terminal b.w. which differed <10% from the study controls).

Data were also presented indicating that Metam-Na induced a thymus weight decrease (–50%, $p<0.001$) occurring in ♀F344-rats treated at 100 mg/kg b.w./d for 7d, thus showing a more severe weight drop than in mice) but the authors also reported that neither spleen weight nor NK cell activity were affected by metam in the rats, contrarily to what was observed in the mouse.

In a comparative study, examining 3 structurally related compounds (Padgett *et al*, 1992), only metam affected spleen and thymus weight, and decreased splenic NK cell activity *in vivo*, indicating that this pattern of immunological effects is not produced by comparable dithiocarbamates. It was also demonstrated that differences in the *in-vivo* potency of these substances did not correlate with their relative cytotoxic potencies *in vitro*.

Myers *et al* (2005) later demonstrated that metam caused an increase in the stress-related corticosterone in blood, and reduced thymocyte cellularity. Blocking corticosterone either by adrenalectomy or by chemically blocking corticosterone synthesis abrogated the thymocytopenia. However, an additional stressor (restraint) did not act additively or synergistically to increase atrophy, and authors therefore deduced that metam causes thymic atrophy by increasing serum corticosterone.

In a follow-up article (Pruett *et al*., 2005), metam was shown to modify dose-dependently both serum and intraperitoneal cytokines in ♀ $B_6C_3F_1$ mice (treated by single gavage at 50, 100, 200 or 300 mg/kg b.w). Cytokine IL-12 was decreased, and IL-10 increased at 50 and 200 mg/kg b.w./d and above respectively (in mice challenged i.v. 10' post-dose to 60 µg bacterial lipopolysaccharide (LPS)/animal). The authors demonstrated that the modification at the top-dose was caused by the inhibition of the cellular signalling MAP-kinases p38 and JNK in peritoneal macrophages, and consequently an increased (IL-10) or decreased (IL-12) mRNA expression.
The main breakdown product MITC was only tested for its capacity to decrease IL-12 levels in serum (at doses of 17 and 45 mg/kg).
The authors suggested that treatment with metam would shift the predominance of T-helper cell from a Th-1 response type (IL-12) to a (lethal) Th-2 response type (IL-10). The exacerbation of asthma in humans, caused by Metam-Na or MITC (Cone, 1994 – see in B.6.9), would be illustrative of a possible strong Th-2 response. Although theoretically possible, it remains questionable whether the used mouse model would be relevant to a human condition where large numbers of normally non-pathogenic bacteria enter the peritoneal cavity from the GIT (contrarily to the opinion of the authors). It was again remarked that the relevance of the effect on survival was measured only at a relatively high toxic doses of metam (200-300 mg/kg), possibly confounding a primary systemic toxicity with an immunological response.
The authors moreover concluded that MITC was mostly responsible for the effects of metam, which may be over-interpretative, as only the IL-12 level was monitored. The authors thus correctly mentioned that *"further studies should demonstrate if there are effects mediated by Metam-Na that are not caused by MITC".*
Finally, it was demonstrated that metam (at 200 or 300 mg/kg) decreased the resistance to *E.coli* induced peritonitis (mortality rate after injection of up to 2×10^9/mouse) within a time-frame (24-48h) in the mouse.

On the basis of the latter observation, Tan and Pruett (2015), investigated the effects of metam on selected parameters of innate immunity and clearance of bacteria (injected *i.p.* at about 2×10^8/mouse) in a mouse model of sepsis. It was confirmed that metam altered innate immunological parameters thought to be important in resistance to sepsis and decreased bacterial clearance through 12h, but these effects were transient and did not significantly alter bacterial clearance or survival at 24h or later, demonstrating that animals may recover from significant transient decreases in pro-inflammatory cytokines and chemokines and in body temperature. The authors however noted that such transient changes were associated with decreased survival time when a higher challenge dose of bacteria was used, indicating that (not surprisingly) the load of bacterial infection was important, but also that the potential effect of metam-induced effect on clearance of micro-organism is threshold-dependent.

From the Pruett-publications and considering other published studies, it was proposed that the immunomodulating action of metam could be explained by (at least) 3 modes of action (Pruett, 2006):

- it could either act as a free radical scavenger (by means of its free S^- group) or promote oxidation by breaking down to form MITC, which can deplete glutathione.
- it is a potent copper chelator and may affect the availability of copper to a number of copper-dependent enzymes (including some signaling molecules).
- it could induce induces a neuroendocrine stress response characterised by elevated serum corticosterone concentrations, which could affect cytokine production.

In this 2006 publication, the authors demonstrated that (N-acetyl-cystein (NAC) pre-treatment exacerbated the metam-induced suppression of IL-12 and induction of IL-10. The addition of copper also suppressed IL-12 but was inactive on IL-10. The role of the stress response was investigated by pretreating mice with antagonists of corticosterone and catecholamines, which partially prevented the action on IL-10 and IL-12 in the peritoneal fluid. The authors thus suggest that all of the proposed mechanisms have some role in the alteration of cytokine production by metam.

In an attempt to better characterise the molecular and genetic mechanisms of action (MoA) further publications were issued by the Pruett team. Metam-induced inhibition of signaling function, were extensively described and discussed (Pruett *et al.,* 2009). In this paper, metam was demonstrated to affect GSH concentration in peritoneal

macrophages, thereby modulating IL-6 and IL-12 production and directly altering LPS-induced IL-6 production. The authors pointed out however that other MoA must also be involved in modulating IL-10 production, and these mechanisms could also play a role in modulation of IL-12 and IL-6. However, the modulation of IL-6 and IL-12 is also consistent with a scenario in which moderate oxidative stress substantially inhibits IL-12 and IL-6 production although they recognised that higher oxidative stress causes less (for IL-6) or no (for IL-12) inhibition and could counteract the effects of metam on both cytokines.

Deng *et al* (2013) highlighted that metam inhibited expression of molecules in both the TRIF-related MyD88-related TLR4 signaling, in line with the formerly observed reduction of circulating cytokines/chemokines.
They also found inhibition of several TLR4-dependent signalling molecules. The transcriptional factor involved in the inhibition of the innate immune response seems to be NF-κB and/or AP-1. From the effect on several gene expressions, the authors suggested several potential other mechanisms that metam might act on, including type I IFN production/signaling but also induction of reproductive hormones such as LH and FSH.

RMS admits that all these gene function may in some way be affected, but it is extremely difficult to fully integrate the existing toxicologically database in their discussed pathways. As is often the case, the recognition of gene-regulation effects (impacting on an extremely complex network of key effects like immunological responses, tissue regeneration, differentiation or apoptosis, and tumour-cell control) may offer (partly) mechanistic explanations for a number of adverse effects, otherwise revealed in the existing toxicity studies, such as signs of oxidative stress in long-term assays, and suspected genotoxic, developmental and carcinogenic findings, and therefore be of major scientific interest.
However, these mechanistic considerations do not necessarily change the main conclusions regarding most relevant NOAEL's and reference values which are deduced from them. In all abovementioned studies, these specific effects occur moreover at relatively high doses, which are covered by the existing guideline toxicity studies. It should be noted that no untoward effects were observed in long-term studies (albeit of course at lower doses) whereby opportunistic infections would have indicated a compromised immunity of the animals.

In conclusion,
For metam, no *in-vivo* humoral immune functionality was affected, but a possible effect on cellular immunity by metam at doses which are not excessively toxic. However, it should be recognised that no untoward effects were observed in long-term studies (albeit of course at lower doses) whereby opportunistic infections would have indicated a compromised immunity of the animals.
Thus, clear-cut demonstration of the absence of an effect on cellular immunity by metam was not provided, also not with the main metabolite MITC, which was tested in ♀$B_6C_3F_1$ mice up to a dose which could have been higher (Weinstein, 2013). In addition, If the test substance has no significant effect on the anti-SRBC assay, a functional test for NK cells may be performed to test for an effect on non-specific immunity.
In Keil, 1996, some immunological effects were probably associated with high-dose toxicity of MITC. It was of note that only thymus (and not spleen) weight was affected, and shifts occurred in the peripheral WBC compartment, but functionality was not impaired, as cellular (NK) immunity remained unaffected.

RMS thus sees only one immunotoxicity endpoint, which is only partially covered by the existing toxicology studies, namely the uncertainty of the effect of **metam** on cell-mediated immunology, for which no guideline study fully excludes its relevance.
Considering the doses where NK immune functionality could be affected by metam (around 200-300 mg/kg b.w./d in a 7-d exposure timespan, either via intranasal or via oral exposure route), it appears that a sufficiently high MoS exists against the reference values of both metam and MITC (0.001-0.004 mg/kg b.w./d), indicating that the potential risk of immunotoxicity is sufficiently covered by the existing studies to agree on an acceptable risk for this endpoint.
Formallyr, this could be considered a data gap, which is not necessarily critical, taking into account the low human health reference values, most likely covering this insufficiently characterised endpoint.

3. Supplementary studies on the active substance

Notifier provided a series of published scientific papers reporting on metam/MITC:

- **mode of action;**
- **immunotoxicity;**
- **endocrine disruption – non EATS-mediated parameters;**
- **neurotoxicity;**
- **toxicokinetics/metabolism;**
- **genotoxicity;**
- **repeated dose toxicity.**

These articles were assessed by the RMS, the conclusions of which were as followed:

●**Mode of action**:

Abdalla, M.Y.; Hassan, I.M.; Mustafa, N.H.; Tahtamouni, L.H.; Ahmad I.M. Total body glutathione depletion induces oxidative stress and disrupts the immune function in mice. Toxicol. & Environment. Chemistry, 2010**,** 1-14 (DRAR: **B.6.8.2.1/01**)

Summary:
In the present study, the effect of total body glutathione (GSH) depletion induced by L-buthionine-(S,R)-sulfoximine (BSO) treatment on the murine immune system were elucidated. BSO was administered via drinking water to BALB/c mice at a concentration of 20 mM for 14 days to induce total body GSH depletion.
A significant decrease in total GSH (TGSH) of the immune organs such as spleen, liver, peritoneal macrophages as well as blood was observed. The proliferative response of splenocytes against different types of mitogens was significantly decreased in GSH-depleted mice compared to controls. The expression of several antioxidant enzymes was studied in both treated and control mice. No significant change was seen in major antioxidant enzymes; manganese superoxide dismutase and catalase. However, the expression of haem oxygenase-1 was increased in both liver and spleen in BSO-treated mice. Total body GSH depletion also increased lipid peroxidation as demonstrated by higher levels of malondialdehyde in BSO-treated mice. All the above-mentioned changes were reversed to near normal 14 days post termination of BSO treatment. These results demonstrate that TGSH depletion induces oxidative stress and adversely affects the function of the murine immune system.

RMS conclusion: BSO inhibits γ-glutamyl-cysteine synthetase, the rate limiting enzyme for the GSH synthesis, which lowers tissue glutathione. The paper shows that BSO caused a total body GSH depletion and disrupted murine immune function, without apparent effect on spleen ultrastructure but with a necrotising effect on liver tissue, especially in the central zone of hepatic lobule. BSO increased haeme oxygenase-1 and lipid peroxidation (TBARS), thus suggesting that oxidative stress may result from GSH depletion. Although the MoAs of metam/MITC and BSO on GSH level are different, it is likely that metam/MITC may also cause oxidative stress.
Higher malondialdehyde (MDA) levels (or thiobarbituric acid-reactive substances-TBARS) were observed by Kassie *et al.* (2001, B.6.8.1.3.3/01) in HepG2 cells exposed to MITC. It is however noted that the TBARS assay is only an indirect indicator test of oxidative stress/ free radicals.
The data described are considered to provide complementary information in order to understand the MoA, also explaining the toxicity profile and the potential genotoxic, developmental, and tumourigenic potential of metam.

Watanabe T.; Sagisaka, H.; Arakawa, S.; Shibaya, Y.; Watanabe, M.; Igarashi, I.; Tanaka; K.; Totsuka, S.; Takasaki, W.; Manabe, S. A novel model of Continuous Depletion of Glutathione in Mice treated with L-Buthionine (S,R)-Sulfoximine The Jour. of Toxicol. Sciences, 2003, Vol. 28, No. 5, 455-469 (DRAR **B.6.8.2.1/02**)

Summary
In the present study, L-Buthionine (S,R)-sulfoximine (BSO, purity >97%), an inhibitor of glutathione (GSH) synthesis, was administered to male B6C3F1 mice via drinking water at concentrations of 0, 5, 10, 20 or 30 mM (equivalent to 0, 252, 536, 995/1072 and 1502 mg/kg bw/day) for 14 days in order to establish an animal model with continuously depleted levels of GSH. No toxicity was observed at 20 mM BSO, even though a significant decrease in liver weight was observed at 30 mM BSO. GSH levels in the liver, kidney, brain, lung, heart, spleen, pancreas, small intestine, large intestine, skeletal muscle, plasma and blood cells from mice given 20 mM of BSO were all less than those from the control mice continuously throughout a 24-h period. The ratios of the GSH levels to that of the control were 46.4% and 16.7% in the liver and kidney, respectively, suggesting a decrease in GSH conjugation activity in vivo by GSH depletion. Liver cytochrome P450 content and UDP-glucuronosyltransferase activity to p-nitrophenol were not influenced by the BSO dosing. To confirm the adequacy of this GSH-depletion model, 0.125 or 0.25% of acetaminophen

(APAP; daily average intake: 196 to 488 mg/kg bw/day) was administered via diet to this model for 14 days. Nine out of the ten mice given both 20 mM (daily average intake: 613 to 843 mg/kg bw/day) BSO and 0.25% APAP died on Day 2, and remarkable necrosis was observed in the hepatocytes and renal tubular epithelium. Moreover, focal necrosis of hepatocytes with proliferation of fibroblasts was observed on Day 15 in some mice co-administered 20 mM BSO and 0.125% APAP. However, no toxicity was observed in mice given APAP alone. Based on these results, a mouse given 20 mM of BSO via drinking water for 14 days was concluded to be an animal model with continuously depleted levels of GSH in various organs without toxicity. This model shows high susceptibility to toxicity induced by chemicals which are metabolised to electrophilic and reactive metabolite(s), such as APAP.

RMS conclusion: agrees that the animal model could be useful for investigation in the field of idiosyncratic drug reaction. This article may be considered as supplemental information.

●Endocrine disruption – non EATS-mediated parameters

Goldman JM.; Stoker, TE.; Cooper, RL.; McElroy, WK.; Hein, JF. Blockade of Ovulation in the Rat by the Fungicide Sodium N-methyldithiocarbamate - Relationship Between Effects on the Luteinising Hormone Surge and Alterations in Hypothalamic Catecholamines. Neurotox. and Teratology 1994, Vol. 16, No. 3, pp. 257-268 (DRAR: **B.6.8.2.3/01**)

Summary
*Sodium N-methyldithiocarbamate (SMD), also known as **metam** sodium, is a commonly employed soil fungicide and nematocide. Structurally related dithiocarbamates have been found to decrease norepinephrine (NE) synthesis by suppressing the activity of dopamine-O-hydroxylase. Because brain hypothalamic catecholamine (CA) activity is involved in generating the pro-estrus afternoon surge in blood luteinising hormone (LH) which stimulates the final stages of ovulation, this study explored the effect of SMD on this hormonal trigger and its relationship to changes in hypothalamic CAs. Ovariectomised, steroid-primed Long-Evans rats showed a dose-related (25-100 mg/kg bw, i.p.) suppression of the surge and a drop in NE when SMD was given at 1100 h, a few h prior to the expected LH rise. The surge effect was reversed by the α-adrenergic agonist cionidine. With cycling rats, a decline with dose (50-300 mg/kg bw, 1300 h, pro-oestrus) was seen in the percentage of ovulating females, with earlier injections (0900 h) being less effective at the highest dose. At all doses, low circulating levels of LH and prolactin at 1600 h suggested either a blockade in the pro-estrus surges of each hormone or a displacement in their time of occurrence.*
Anterior and posterior hypothalamic NE fell by 3 h post-injection and was accompanied by a rise in dopamine, while serotonin was unchanged. Although there was a distinct parallel between the alterations in regional CAs and the incidence of ovulation at the high doses of SMD, the relationship did not hold as the dose decreased. A similar dissociation between ovulation and CAs was seen when equimolar doses of SMD or methylisothiocyanate, a principal metabolite, were given by gavage. At the regional level of analysis employed, the data indicate that while IP injections of SMD are able to block the LH surge and ovulation in these rats, the dose-response relationship suggests that, along with induced alterations in CA metabolism, an additional factor may be involved in the observed effects.

RMS conclusion: in this study, signs of systemic toxicity were not reported and it is thus difficult to separate primary adverse ED effects from secondary effects related to systemic toxicity, consecutive to the high doses used.
However, it is plausible that this study is of uncertain toxicological relevance, as the doses used here are quite high and expected to be systemically toxic.
In addition, it is noted that neither metam nor MITC display overt adverse fertility findings in multigenerational studies conducted with intact rats where the a.s. or its main metabolite were administered for a continuous period.

On the other hand, the endpoints investigated in this study were not examined in other studies provided by the notifier according to the ongoing guidelines and it is therefore difficult to exclude an effect in regulatory studies. The authors point out that there may be a class effect of dithiocarbamates. Substances as disulfiram (tetraethylthiuram disulfide) and its metabolite diethyldithiocarbamate (DEDC), can interfere with catecholamine neurotransmitter metabolism by inhibiting the activity of dopamine-β-hydroxylase through a chelation of the Cu-containing portion of the hydrolase. As a consequence, the enzymatic conversion of dopamine (DA) to norepinephrine (NE) is suppressed. Therefore, is seems realistic that members of this class of compounds could interfere with physiological processes that depend on well-known catecholaminergic mechanisms of neurotransmission. The authors infer that dithiocarbamate administered during a sensitive period can suppress a pituitary LH surge through such an interference with catecholamine activity, and potentially block oocyte release.

Discrepancies between the two routes of metam administration in the incidence of ovulation are interpreted as being due to differences in breakdown or absorption of the compound. Although the level may still have been able to induce significant changes in catecholamine concentrations, it may have been insufficient to block the LH surge and oocyte release after oral administration. The authors think that for the doses tested, the observed ovulatory effects are dependent on the route of administration, unlike the changes in hypothalamic catecholamines, which suggests that an additional factor is involved in these observed effects. This is possible, but more recent publications on the

identification of such a factor have not been found.

While considerations of non-EATS modalities are outside the scope of the current ED assessment, and so far no overt ED effects have been identified, neuro-endocrine findings should be subject of a further follow-up in the future assessment of dithiocarbamates, including metam and its main metabolite MITC.

Goldman JM, Cooper RL, Murr AS. Reproductive functions and hypothalamic catecholamines in response to the soil fumigant metam sodium: Adaptations to extended exposures. Neurotoxicology and Teratology 29 (2007) 368-376 (DRAR: **B.6.8.2.3/02**).

Summary
Metam *sodium (MS) is a soil fumigant and Category II pesticide with a relatively low toxicity in mammals. Previous data have shown an ability to impair reproductive mechanisms in ovariectomized, estradiol-primed rats. A single i.p. injection blocked the luteinizing hormone (LH) surge that in gonadal-intact females initiates the final stages of follicular and oocytic maturation and serves as the trigger for ovulation. The effect paralleled a fall in hypothalamic norepinephrine (NE) and rise in hypothalamic dopamine (DA) that was likely due to suppression in dopamine- β-hydroxylase activity. In addition to determining the influence on catecholamine (CA) concentrations from a single oral exposure to MS, the present study explored effects of longer, 3-week treatments on estrous cyclicity, the LH surge, ovulation and hypothalamic CAs. Normally cycling SD rats were administered MS (0–200 mg/kg/d, oral) and cyclicity was monitored daily. At the end of the 3rd week, proestrous blood was sampled over the afternoon from regular 4-day cyclers for a determination of LH. These animals were then killed on the following day of estrus (treatment days 21–26) for oocyte retrieval and assessment of hypothalamic CAs. Results showed that shortly after treatment began there occurred a dose-related period of persistent diestrus that typically lasted 8–16 d before regular cycles were reinstated. After 3 weeks, no effects were seen on the magnitude/timing of the LH surge or ovulated oocyte numbers. Anterior and posterior hypothalamic NE and DA were not significantly different from controls, although DA turnover (reflected by the ratio of DOPAC {3,4-dihydroxy-phenylacetic acid} to DA) in both anterior hypothalamic and caudate regions was decreased at all dosages. The data indicate that a 3 week oral exposure to MS induced an initial period of extended diestrus before the resumption of apparently normal reproductive activity, with previously reported CA alterations (apart from a persistent alteration in the DOPAC/DA ratio) being normalized by the end of dosing.*

RMS conclusion: in this study, a transient influence of metam on the oestrous cycle was observed.
With regards to the doses applied, disturbance of the general metabolic capacity can be assumed.
It is however noted that in two-generation study of MITC ([redacted] 2014, **B.6.8.1.5.1/02**), an increase in oestrous cycle length was observed at top dose (20 ppm) in bith F_0 (+10%) and the F_1 (+20%) generation, although it was in the HCD.
The oestrous cycle was not evaluated in the two other sexual function and fertility studies with MITC ([redacted], 1987, **B.6.8.1.5.1/01** and [redacted] 2013, **B.6.8.1.5.1/03**), nor in the metam multigeneration study ([redacted], 1993, **B.6.6.1/01**).

●Neurotoxicity

Vaccari, A. Dithiocarbamate Pesticides Affect Glutamate Transport in Brain Synaptic Vesicles. The Journal of Pharmacology and Experimental Therapeutics (1999) JPET 288:1-5 (DRAR : **B.6.8.2.4/01**).

Summary
Dithiocarbamate *compounds are widely used agricultural fungicides that display low acute toxicity in mammals and that may become neurotoxic after prolonged exposure. Mancozeb, among other dithiocarbamates tested, proved to be the most potent (Ki = 0.27 mM) at noncompetitively inhibiting the in vitro ATP-dependent uptake of [^{3}H]glutamate in rat cortical vesicles. Furthermore, mancozeb partially (20%) inhibited the ATP-dependent uptake of [^{14}C]methylamine, used as an index for the vesicular transmembrane proton gradient (ΔpH), and evoked its efflux from organelles previously incubated with the 3H-labeled marker. Meanwhile, the vesicular uptake of 36chloride- anions whose concentrations regulate the transmembrane potential gradient (ΔψSV) was not impaired. The dithiocarbamate effects on the vesicular transport of [3H]glutamate thus appeared to involve mainly the ΔpH gradient rather than the potential gradient. Dithiocarbamate metabolites, the potent neurotoxin carbon disulfide included, did not affect the uptake process, thus implying the relevance for inhibition of the persistence, if any, of parent compounds in the brain. The present novel and potent in vitro interferences of selected dithiocarbamate pesticides with the vesicular transport of glutamate, if representative of in vivo alterations, may play some role in the probably complex origin of dithiocarbamate neurotoxicity. However, the dithiocarbamate metam (named metham in the publication) and the dithiocarbamate metabolite carbon disulfide did not affect the glutamate transport in brain synaptic vesicles.*

RMS conclusion: as mentioned by the applicant, dithiocarbamates are known to provoke a wide range of neurobehavioural effects, including ataxia, hindlimb paralysis, hemiparesis, convulsions, behavioral abnormalities, and neuropathological changes in the brain. In the present study, however, it can be concluded that metam and its metabolite carbon disulfide did not affect the glutamate transport in brain synaptic vesicles.

Barlow, KB.; Thiruchelvam, MJ.; Bennice, L.; Cory-Slechta, DA.; Ballatori, N.; Richfield, EK. Increased synaptosomal dopamine content and brain concentration of paraquat produced by selective dithiocarbamates. Jour. of Neurochemistry 2003, 85, 1075-1086 (DRAR: **B.6.8.2.4/02**)

Summary
*Exposure to pesticides may be a risk factor for Parkinson's disease based on epidemiologic data in humans, animal models and in vitro studies. Different dithiocarbamate pesticides potentiate the toxicity of both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and paraquat in mouse models of Parkinsonism by an unknown mechanism. This study examined the effects of commercially used dithiocarbamates on [^{3}H]dopamine transport in striatal synaptosomal vesicles and on the concentration of [^{14}C]paraquat in vivo in mice. Different ethylenebis-dithiocarbamates and diethyl-dithiocarbamate increased dopamine accumulation in synaptosomes, whereas dimethyl-dithiocarbamate and **methyl-dithiocarbamate** did not. Increased dopamine accumulation in synaptosomes was dose dependent and was related to the carbon backbone of these molecules. The dithiocarbamates that increased accumulation of dopamine did not alter the influx of dopamine, but rather delayed the efflux out of synaptosomes. These same dithiocarbamates also increased the tissue content of [^{14}C]paraquat in vivo by a mechanism that appeared to be distinct from the dopamine transporter. There was a consistent relationship between the dithiocarbamates that increased synaptosomal accumulation of dopamine and tissue content of paraquat, with those previously demonstrated to enhance paraquat toxicity in vivo. These results suggest that selective dithiocarbamates may alter the kinetics of different endogenous and exogenous compounds to enhance their neurotoxicity.*

RMS conclusion: regarding metam, the present study indicates that it did not significantly increase the synaptosomal dopamine content.

Kaikai, NE. *et al* Prenatal exposure to the pesticide metam sodium induces sensorimotor and neurobehavioral abnormalities in mice offspring.
Environ Toxicol Pharmacol. 2020 Feb;74:103309 (DRAR : **B.6.8.2.4/03**)

Summary
The present study has investigated developmental neurotoxicity of Metam sodium (MS), from gestational day 6 and throughout the gestation period until delivery. Therefore, mated female mice were orally exposed on a daily basis to 0 (control), 50, 100 or 150 mg of MS/kg of body weight and their standard fertility and reproductive parameters were assessed. The offspring were examined for their sensorimotor development, depression and cognitive performance. Our results showed that MS exposure during pregnancy led to one case of mortality, two cases of abortion and disturbed fertility and reproductive parameters in pregnant dams. In offspring, MS induced an overall delay in innate reflexes and sensorimotor performances. Furthermore, all prenatally treated animals showed an increased level of depression-like behavior as well as a pronounced cognitive impairment in adulthood. These results demonstrated that prenatal exposure to MS causes a long-lasting developmental neurotoxicity and alters a wide range of behavioral functions in mice.

Kaikai, NE. *et al*. Metam sodium exposure during pregnancy and lactation in mice caused behavioral abnormalities and oxidative stress in offspring
Environ Toxicol Pharmacol. 2021 Jul;85:103630, (DRAR : B.6.8.2.4/04)

Summary
Metam sodium (MS) is a widespread biocide with a broad-spectrum activity. Here, we addressed the behavioral impact of MS by exposing female mice to 50, 100 and 150 mg/kg of MS during both pregnancy and lactation, and evaluated the oxidative stress as a potential mechanism of MS-induced neurotoxicity. The results showed that MS affected fertility and reproduction parameters as well as some aspects of maternal behavior, especially at high doses. In offspring, MS caused a significant delay in the ontogeny of sensorimotor functions. In addition, treated mice exhibited during adulthood an increase of anxiety-like, depression-like behaviors as well as learning and memory impairment. These alterations were accompanied by an increase of the superoxide dismutase activity, and a significant decreased catalase and malondialdehyde activities in specific brain areas. The present work revealed that early exposure to MS induced sensorimotor and behavioral impairments in offspring likely associated with onset of oxidative stress.

Summary and conclusion of the published studies on metam/MITC developmental neurotoxicity

(i) In a published developmental neurotoxicity gavage study (Kaikai, 2020) performed on Swiss mice, exposed prenatally at 0, 50, 100 and 150 mg/kg bw./d from GD6 to GD21 (gestation), MTD seems exceeded at the top-dose, based upon one fatality and abortion in 2/7 dams, as well as the slightly prolonged gestation period.
Regarding developmental neurotoxicity endpoints, most sensimotoric adverse findings in the young pups were observed at 100 mg/kg bw/d onwards, although increased incidences of impaired cliff avoidance ability were observed at the lowest dose of 50 mg/kg b.w./d onwards. Negative geotaxis reflex was also impaired in pups on PND10, but not (significantly) on PND12 at the lowest dose (although a slight trend was present).
In the adult offspring, the treatment was without observable effect on locomotor functions, and also the Y-maze performance scores were unremarkable when compared to the control group. However, one learning/memory function (passive avoidance test) scored lower in the exposed rats, and the retention of the avoidance performance tested more poorly 24h than 2h after the shock exposure.

(ii) A follow-up was published in a second NDT gavage study (Kaikai, 2021) on the same strain and at the same doses but in a wider administration scheme. In this study, mice were exposed perinatally at 0, 50, 100 and 150 mg/kg bw/d from GD6 to PND21 (gestation+lactation).
MTD seemed exceeded at 100 mg/kg bw/d and above, owing to mortality of one dam at 100 mg/kg b.w./d and 4 dams at the top-dose. Maternal behaviour was affected at the two highest doses, owing to the impaired nest building and pup retrieving activities.
Pup DNT findings. Surface righting time seemed to be increased at 100 mg/kg bw/d and above at PND7-9. Cliff avoidance time increased at 50 mg/kg bw/d onwards. The mouse pups, when placed on an inclined plane (negative geotaxis) returned more slowly uphill when treated at 50 mg/kg and above *vs* controls at PND10, but the dose-response was unclear on PND12. Finally, the pups fell down the rotarod more quickly at 100 mg bw/d and above in trial 1 but only at top-dose in trial 2. Overall, pup NT findings were slightly altered at the lowest dose, but most findings were restricted to the mid- and top-dose.
At adulthood, most DNT findings related to « anxiety-like behaviour» were significant at the two highest dose-groups, when compared to the control group. The difference between the lowest dose-group *vs* control is debatable, although it may be remarked that overall, a slight effect trend existed, indicating a plausible treatment-relationship. For most effects related to «depression-like behaviour», endpoints were significantly altered at 50 mg/kg b.w./d and above. Also here it may be remarked that overall, a slight effect trend exists for most endpoints, although less marked than for the former endpoints «anxiety-like behaviour».
The authors performed an analysis of oxidative stress parameters, including SOD and CAT activities and MDA dosage in CNS homogenates. The treatment demonstrated no convincing effect on SOD, CAT activity was decreased at 50 mg/kg bw/d and above, and MDA was decreased (the latter being contra-intuitive, under the hypothesis of a treatment-related oxidative stress).

RMS considered that the observations under the conditions of these 2 experiments should be taken with reservation, taking into consideration the deviations from the OECD test guideline TG 426. These deviations included: insufficient n° of animals per dose group, non-transparent allocation of dams in the different dose-groups, uncertainty about the test article intake (in the absence of its analysis in the gavage solution), incomplete assessment of systemic toxicity, (like body weight, food consumption), no neuropathological evaluation of CNS, uncertain internal test protocol validation.
Therefore, the studies are considered acceptable with restrictions, and are considered to provide complementary information only.

Following overall NOAELS could tentatively and precautionously be derived:
-the **maternal toxicity NOAEL** = **50 mg/kg b.w./d**. (based upon mortality at higher doses)
-the **neurodevelopmental NOAEL** would tentatively be set **<50 mg/kg b.w./d**,
This is based upon limited indications of affected sensimotor functions in young pups and of learning/memory functions in adult offspring, thus lower than the maternotoxic NOAEL.

The doses used in mice are relatively high (50, 100, 150 mg/kg bw), compared to those used in either rat and rabbit regulatory developmental toxicity studies (varying from 5-60, 20-80, 10-100/120 mg/kg bw.w/d), but where developmental NOAEL's were generally equal to or higher than the parental NOAEL and overall ≤40 mg/kg b.w./d. Here, the lowest dose (50 mg/kg bw/d) is higher than these values. **RMS** also remarks that only a limited number of DNT endpoints were affected at the lowest dose of 50 mg/kg bw/d, and that most DNT effects in the published studies were seen at 100 mg/kg bw/d and above, and equivalent to the dose causing overt maternotoxicity.

Due to the high doses used, it is difficult to conclude if the effects described in this study are reflecting a direct neurotoxic action of metam or are secondary to systemic toxicity, even at the lowest dose tested. In the guideline 90d rat NT study ([redacted], 1994, B.6.7.1/03), the tested doses varied between about 2-15 mg/kg b.w./d, with a systemic toxicity NOAEL of <2 mg/kg b.w./d, and a NT NOAEL of 15 mg/kg b.w./d.

On the other hand, these studies were conducted on mice (although there is also no indication that among rodents, mice would be particularly more sensitive to the toxic action of metam than the rats), and it should be acknowledged that no regulatory counterpart on this specific DNT study exists in any species.

Although this published study is considered complementary information only, the question could be asked whether a DNT would constitute a data gap.
In the opinion of the **RMS**, and considering animal welfare considerations, it may be accepted that the existing developmental studies in particular, and the overall most relevant NOAEL in general, are sufficient to cover a potential developmental NT effect, also taking into consideration that neurotoxic effects are probably not the most critical effects of metam/MITC, and therefore no further study should be conducted on neither metam nor MITC.

●Toxicokinetics/metabolism

Moorhouse, K.G. and Casida, J.E. Disparate effects of representative dithiocarbamates on selected immunological parameters in vivo and cell survival in vitro in female B6C3F1 mice. Pesticide Biochem. and Physiology 1992, 44, 83-90 (DRAR: **B.6.8.2.5/01**)
Summary
Selected sulfhydryl-reactive pesticides activate mouse liver microsomal glutathione S-transferase (GSTm) assayed in purified form free from cytosolic transferases (GSTcs) with 1-chloro-2,4- dinitrobenzene as the substrate. Maximum activations were as follows: 1315% for N-ethylmaleimide (positive control) at 1 mM, 374% for chloranil at 0.01 mM, 272% for EPTC sulfoxide at 10 mM, 255% for captan at 0.1 mM, 228% for acrolein at 1 mM, and 152% for methyl isothiocyanate at 10 mM. Pesticides which showed little or no activation were alachlor, atrazine, tetramethrin, and tridiphane. The toxicological relevance of GSTm activation was examined with the aforementioned activators plus EPTC and allyl alcohol. Experiments were conducted in vitro or in vivo with washed microsomes (which contain not only GSTm but also some GSTcs) and the postmitochondrial supernatant fraction with its high GSTc activity. For the in vivo eyperiments mice were administered intraperitorially at 100 mg/kg bw. These studies established that GSTm either inactivated or activated, was not a significant contributor to overall mouse liver GST activity.

RMS conclusion: It is shown here that microsomal glutathione S-transferase (GSTm) is not a significant contributor to overall mouse liver GST activity.

Schmidt RJ. and Chung LY. Perturbation of glutathione status and generation of oxidative stress in mouse skin following application of contact allergenic sesquiterpene lactones and isothiocyanates. Xenobiotica, 23:8, 889-897 (DRAR: **B.6.8.2.5/02**)
Summary
Female WSP mice were exposed to each sesquiterpene lactone or isothiocyanate. The applications were made to the inner surface of one ear of each mouse and reactions were read after a further 24, 48, and 72 h.
Results from sensitisation and cross-sensitisation experiments established that the mouse was immunologically responsive to the two classes of xenobiotic under investigation (i.e. sesquiterpene lactones and isothiocyanates). Although the procedure used was suboptimal for detecting weak sensitisers, only methyl and phenyl isothiocyanates produced equivocal reactions. Nevertheless, a clear correlation appeared to exist between sensitising activity and ability to perturb glutathione status in mouse skin 12h after exposure to xenobiotic.
It was concluded that sensitising sesquiterpene lactones and isothiocyanates could induce oxidative stress in mouse skin, possibly as a result of their reductive metabolism.

RMS conclusion: The results of the study can be used to support the mode of action of GSH depletion and subsequent oxidative stress induction after metam/MITC exposure.

Tynes R.E. and Hodgson E. Magnitude of Involvement of the Mammalian Flavin-Containing Monooxygenase in the Microsomal Oxidation of Pesticides. J. Agrie. Food Chem. 1985, 33, 471-479 (DRAR: **B.6.8.2.5/03**)
Summary
The oxidation of sulfide-containing organophosphate and carbamate pesticides by the flavin-containing monooxygenase has been measured in mammalian microsomes made devoid of cytochrome P-450-dependent activity, primarily through the use of inhibitory antibodies against NADPH-cytochrome P-450 reductase. Rates of metabolism were determined for mouse, rabbit, and rat liver, lung, and kidney microsomes and for pig liver microsomes. Substrate specificity of the enzyme in different species and tissues is similar. Lung and kidney microsomes have high flavin-containing monooxygenase levels, and this enzyme is important relative to cytochrome P-450 in these tissues. Thioether-containing organophosphates are effective substrates for the flavin-containing monooxygenase in mouse liver microsomes, with Km values between 3.5 and 36 μM. Thioether-containing carbamates are less effective substrates, having Km values near 280 μM. Other substances oxidized include (methylthio)phenyl-containing

organophosphates, certain phosphonodithioate pesticides, certain dithiocarbamate soil fumigants, ethylenethiourea, nicotine, selenourea, and diethylphenylphosphine.

RMS conclusion: dithiocarbamates are rather poor substrates for both the pig and mouse liver flavin monooxygenases.

Lee MS. Enzyme induction and comparative oxidative desulfuration of isothiocyanates to isocyanates. Chem. Res. Toxicol. 1996, 9, 1072-1078 (DRAR: **B.6.8.2.5/04**)

Summary

Enzyme induction of oxidative metabolism of isothiocyanates to isocyanates by rat liver microsomes and comparative metabolic conversion of some isothiocyanates were investigated. Metabolic activity was assayed by trapping the isocyanate metabolites from isothiocyanates with the inclusion of 2-aminofluorene to form the respective mixed ureas as previously described for the 2-naphthyl isothiocyanate. Male F344 rats were fed either a conventional grain diet for induction with Aroclor 1254 or AIN 76A diet without antioxidant beginning 2 weeks before treatment with Aroclor 1254, â-naphthoflavone, isosafrole, or phenobarbital. Enzymes responsible for the metabolism of 1- and 2-naphthyl isothiocyanate were inducible by all four agents, Aroclor being the best under the current induction protocol and metabolic conversion assay procedure. On the other hand, enzymes responsible for the metabolism of benzyl isothiocyanate were induced only by Aroclor and, to a lesser extent, by phenobarbital. For the comparative metabolic conversion studies, using the microsomes from Aroclor-treated rats fed a conventional grain diet, the rates of metabolic conversion followed the order of 1-naphthyl >> phenyl > benzyl and phenethyl >> propyl, ethyl, and methyl isothiocyanates.

RMS conclusion: the conversion of isothiocyanates to isocyanates is mediated by P450 enzymes and not by the flavin-containing monooxygenase, since these reactions are inhibitable by CO and heating of microsomes did not decrease the product formations.

The data provide some insight in the metabolism of isothiocyanates. The data ced as supporting data but have no effect on the risk assessment.

Munday, R.; Zhang, Y.; Fahey, JW.; Jobson, HE.; Munday, CM.; Li, J.; Stephenson, KK. Evaluation of Isothiocyanates as Potent Inducers of Carcinogen-Detoxifying Enzymes in the Urinary Bladder: Critical Nature of In Vivo Bioassay. Nutrition and Cancer 2006, 54(2), 223-231 (DRAR: **B.6.8.2.5/05**)

Summary

Deficiency of carcinogen-detoxifying phase 2 enzymes, such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), increases bladder cancer risk in humans. In this study the GST and NQO1 were investigated after treatment with isothiocyanates (ITCs) in cell lines (in vitro: rat bladder carcinoma NBT-II cells, human bladder carcinoma UM-UC-3 cells, and murine hepatoma Hepa1c1c7 cells) and in Sprague-Dawley rats in vivo). Additionally the urinary excretion of selected ITCs were examined in rats.

Several ITCs that have not been previously examined 1-methylbutyl ITC in particular potently and preferentially induce both GST and NQO1 in the rat bladder. Comparison of 25 ITCs that are closely related in chemical structures showed that a 3–5-carbon aliphatic side chain with a methyl group attached to the alpha carbon was crucial for maximal inducer activity in the bladder. Surprisingly, cell-based bioassays failed to predict the phase 2 enzyme-inducing activity of the ITCs in the bladder. Furthermore, although ITCs are principally metabolised in vivo to dithiocarbamates (DTCs), which are believed to serve as the carriers of ITCs and are rapidly eliminated and concentrated in the urine, the total urinary levels of ITC plus DTC did not correlate with the degree of GST and NQO1 induction by the ITCs in the bladder of rats. Thus, several underappreciated ITCs are exceedingly potent inducers of GST and NQO1 in the rat bladder but were predicted neither by in vitro bioassays of phase 2 enzyme induction nor by their appearance or concentration in urine in vivo.

RMS conclusion: several underappreciated ITCs are exceedingly potent inducers of GST and NQO1 in the rat bladder but were predicted neither by in vitro bioassays of phase 2 enzyme induction nor by their appearance or concentration in urine in vivo.

The results of the study can be used as supportive information on metabolism. The results are not relevant for the hazard and risk assessment.

Zendzian, RP. Pesticide Residue on/in the Washed Skin and its Potential Contribution to Dermal Toxicity. Jour. of Applied Toxicology 2003, 23, 121-136 (DRAR: **B.6.8.2.5/06**)

Summary

Washing the skin of humans or experimental animals after exposure to a pesticide or other chemical may leave a major portion of the dose on/in the washed skin. Questions have been raised as to whether this skin residue can contribute to the toxicity of a pesticide by continued post-wash absorption. This review article summarizes different studies in rats, where exposure with and without washing the skin was performed. In a set of 19 pesticides tested in the rat to determine the fate of this skin residue, absorption from the washed skin continued in 15 at all doses tested, continued in 2 pesticides at only some of the doses tested and did not continue in 2 volatile pesticides. However, only nine pesticides

showed an increase in systemic concentration following absorption from the washed skin, which can be considered indicative of potential increased toxicity. The time of occurrence and magnitude of the increase varied with chemical and dose, being a combination of rate and magnitude of absorption and rate and magnitude of excretion of the absorbed chemical. Similar patterns of continued absorption of skin residue may be expected to occur in humans.

RMS conclusion: it is shown in the study that for 9/19 pesticides ((i.a. metam sodium) the systemic dose increased following the skin wash. In these chemicals the skin residue can be expected to increase toxicity. A problem that occurred when different studies were compared is the unstandardised sacrifice intervals.
The results of the study can be used as supportive information on dermal absorption. The results are not relevant for the hazard and risk assessment.

Lam, W.W. et al. Metabolism in rats and mice of the soil fumigants of metam, methyl isothiocyanate and Dazomet. J.Agric.Food Chem., 41, 1497-1502 (DRAR **B.6.8.5.2/07**)
Summary
Isotopic labelling of metam, methyl isothiocyanate (MITC), and dazomet with 13CH3 and 14CH3 and of metam and MITC with 13C=S provided the materials for metabolite identification by 13C NMR and quantitation by HPLC analysis and radiocarbon counting. Rats and mice were treated intraperitoneally and the metabolites studied at 48 h. Most of the 14CH3 label for each compound in mice appears in urine (58-80%) or is retained in the body (8-12%), particularly the liver and kidney. The major metabolites in each case from rats are S-(IV-methylthiocarbamoyl)glutathione in the bile and S-(A*-methylthiocarbamoyl)mercapturic acid in the urine, whereas from mice the mercapturate is a minor metabolite. Methylamine is a major urinary component following treatment with methylamine or dazomet (rats and mice) or metam (mice) but not with MITC. Detoxification by conjugation with glutathione (GSH) appears to involve direct reaction for MITC, GSH S-transferase-catalyzed reaction for metam, and the intermediacy of either MITC or metam for dazomet.

RMS conclusion: After ip administration of metam, MITC or methylamine, radioactivity was found mainly in urine. Fecal excretion was low. A small part of the radioactivity was exhaled as 14CO2. The recovery was insufficient in the mice treated with metam and methylamine, which was explained by the authors by the possibility that not all expired radioactivity was trapped. About 85-90% of the dose of the carcass radioactivity was not extractible with MeOH, suggesting a covalent binding to the biological macromolecules of the matrix. Radioactivity was widely distributed among the tissues, with a preference for liver, lung and kidney. At 48h, the average level of tissue radioactivity was 10% (MITC)-30% (methylamine) of the dose.
A principal detoxification step for metam and MITC is their conversion to the GSH conjugate. This reaction occurs without GST for MITC, while metam requires the enzyme. This reaction results ultimately in a large amount of mercapturate in urine with methylamine as additional product. Metabolism of methylamine could give rise to formaldehyde and according to the authors, 14C residues in tissue could derive from extensive degradation to formaldehyde and formic acid.
The results presented in table 5.8.2-38 show that, although rat and mice have several metabolites in common, metabolism differs quantitatively and qualitatively. There are large amounts of unidentified metabolites, particularly in mice. The vast majority of the unknown 14C is very polar.
The paper is considered supporting information for the toxicokinetics of metam and MITC as well as for the mode of action discussion.

●Genotoxicity

Kligerman, A.D. . An Evaluation of 25 Selected ToxCast Chemicals in Medium-Throughput Assays to Detect Genotoxicity. Environmental and Molecular Mutagenesis 56:468^476 (2015) (DRAR: **B.6.8.2.6/01**)
Summary
S. typhimurium strains TA 98, and mixed Ames II strains (TAMix), that consists of TA7001, TA7002, TA7003, TA7004, TA7005, and TA7006 combined into a single culture were exposed with Methyl isothiocyanate (MITC) in the presence and absence of metabolic activation for 48 hours. Vehicle and positive controls were included in each experiment. In the Ames test the compound was tested eight concentrations ranging from 6.25 to 800 µg/plate with and without S9 mix (Aroclor 1254-induced rat liver S9 fraction).
No data about precipitation of the test substance was reported. No data on bacteriotoxic effects was provided.
MITC was not found to be mutagenic in the Ames II Assay either without S9 mix or after the addition of a metabolising system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
Based on the results of the present study, the Metam Sodium is not mutagenic in the Ames II Assay with and without metabolic activation under the experimental conditions chosen.

RMS conclusion: in this study, the authors tested 25 chemicals using two ToxCast Medium-Throughput Genotoxicity (MTG) assays: the “Ames II” assay and the “96-well In Vitro MicroFlow(®) Micronucleus (MN)” assay (all both with

and without S9 activation). According to the authors, the Ames II assay showed a reasonable correlation with published Ames test data and industry submissions and overall concordance was 73% both with and without metabolic activation. The flow MN assay had concordances of 71% and 58% with and without metabolic activation, respectively, when compared to published data and submissions. Under the experimental conditions chosen, MITC was not mutagenic in the Ames II Assay with and without metabolic activation. In the In Vitro MicroFlow® Micronucleus assay, the MITC was not considered to possess either chromosome-damaging (clastogenic) activity or to induce numerical chromosomal aberrations (aneugenic activity) under in vitro conditions in CHO-K1 cells in the presence or absence of metabolic activation.
These data are considered reliable with restriction providing supportive information on the mutagenicity of MITC in an Ames test and micronucleus test.

Knight, A.W. et al. Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast chemicals Regulatory Toxicology and Pharmacology 55 (2009) 188–199 (DRAR: **B.6.8.2.6/02**)
Summary
Three high-throughput screening (HTS) genotoxicity assays-GreenScreen HC GADD45a-GFP (Gentronix Ltd.), CellCiphr p53 (Cellumen Inc.) and CellSensor p53RE-bla (Invitrogen Corp.)-were used to analyse the collection of 320 predominantly pesticide active compounds being tested in Phase I of US. Environmental Protection Agency's ToxCast research project. Between 9% and 12% of compounds were positive for genotoxicity in the assays. However, results of the varied tests only partially overlapped, suggesting a strategy of combining data from a battery of assays. The HTS results were compared to mutagenicity (Ames) and animal tumorigenicity data. Overall, the HTS assays demonstrated low sensitivity for rodent tumorigens, likely due to: screening at a low concentration, coverage of selected genotoxic mechanisms, lack of metabolic activation and difficulty detecting non-genotoxic carcinogens. Conversely, HTS results demonstrated high specificity, >88%. Overall concordance of the HTS assays with tumorigenicity data was low, around 50% for all tumorigens, but increased to 74-78% (vs. 60% for Ames) for those compounds producing tumours in rodents at multiple sites and, thus, more likely genotoxic carcinogens. The aim of the present study was to evaluate the utility of HTS assays to identify potential genotoxicity hazard in the larger context of the ToxCast program, to aid prioritization of environmentally relevant chemicals for further testing and assessment of carcinogenicity risk to humans.
For metam negative results were observed in all three screening assays, indicating that metam has no genotoxic properties supporting available in vitro and in vivo data.

RMS conclusion: under the experimental conditions chosen, it may be concluded from the three assays that metam is not mutagenic. The paper is considered as supportive information.

●Repeated dose toxicity

Jonderko, Gerard; Kita, Izabela; Kita, Kazimierz; Knapek, Roman. Evaluation of subchronic toxicity of carbothion (PF-70), a new fungicide for corn protection. Investigations carried out on rats. Part I. General condition of animals and pathomorphological findings in internal organs. Annales Academiae Medicae Silesiensis, (1990) Vol. 20, pp. 13-17, 5 plates (DRAR: **B.6.8.2.7/01**)

Jonderko, Gerard; Kobes, Stanislaw; Knapek, Roman; Lachowicz, Krzysztof. Evaluation of subchronic toxicity of carbothion (PF-70), a new fungicide for corn protection. Investigations carried out on rats. Part II. Effect on some hematologic and biochemical parameters in blood serum and urine. Annales Academiae Medicae Silesiensis, (1990) Vol. 20, pp. 19-31 (DRAR: **B.6.8.2.7/02**)

Summary
Groups of 8 male and 8 female rats were treated with the following concentrations of the preparation: 0, 100, 400, 1600 and 6400 mg/kg of food for 13 weeks. The dose in mg/kg bw was not given in the publication. The dose was therefore calculated for this evaluation using the mean food consumption and the body weight at the beginning and end of the study. The range of doses is provided below.

Concentration in food [mg/kg]	**Dose [mg/kg bw/d]**	
	Male	**Female**
100	0.006-0.020	0.007-0.019
400	0.023-0.078	0.030-0.076
1600	0.101-0.343	0.119-0.300
6400	0.426-1.412	0.467-1.110

The two high doses caused, principally in males an increased food intake. A significant weight loss was observed in females at 6400 mg/kg food. In females the intoxication caused a dose dependant increase of the hypophysis mass beginning from the concentration of 100 mg/kg food. Also in females a significant increase of thyroid mass was found at the concentration of 6400 mg/kg food. No pathomorphological alterations in parenchymatous organs were observed. The obtained results allow to conclude that the highest tolerable Karbation concentration is below 100 mg/kg food.

No adverse effects were observed on haematology as well as clinical chemistry. Red and white blood cell parameters were not affected. Clinical chemistry parameters in serum or urinalysis did not indicate damage of liver or kidney.

RMS conclusion:
Because of the scarce information on test substance and material and methods section the data were not used for the risk assessment.
(Note: carbothion is another name for metam sodium)

Please note that, following the implementation of the scientific criteria for the determination of endocrine disrupting properties introduced by regulation (EU) no 2018/605, the endocrine assessment for both humans and the environment should be presented under chapter 2.10.

2.6.9 Summary of medical data and information

2.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Medical surveillance in the plant
Metam is not the only plant protection product that is manufactured or handled at the production plant. However, all plant protection products concerned are based on active substances from the dithiocarbamate group. Medical surveillance consists of taking blood samples (RBC, Hb, Hct, MCV, MCH, WBC and formula, GOT, GTP, g-GT, AF, creatinine, TSH, T4) and urine samples (TTCA, thiothiazolidine-4-carboxylic acid, Zn, Mn) at yearly intervals. Next to this, tests for lung function are performed (Vital capacity and Forced Expiratory Volume in one second).
All personnel active on the dithiocarbamate plant are seen by the physician on site for a yearly clinical investigation with specific attention for complaints (skin irritation, allergic reactions of skin and lungs, others). During this periodic visit, attention will be made on the correct use of collective and personal protective equipment.
All personnel working in warehouse, crop protection production, shipping and laboratory with a career for at least 1 day to 40 years are followed by the medical section for medical surveillance (Belgian legislation). Blood samples showed no systematic deviations (see clinical tests below). 3 cases of proven skin allergic reaction (sensitisation) have been identified, which were followed by an internal mutation to another workplace within the plant. Skin irritation is seen sporadically in the plant (difficult to explore which product is on the origin, correct use of personal equipment), no other symptoms/health effects have been recorded.

Clinical tests (biological monitoring)
Urine samples from the exposed workers should be taken by 'end of the shift' the same day or 3 days after the shift, as the elimination (excretion of metabolites) happens in 2 phases in the urine: after 2-6h and 65-70h. When handling dithiocarbamate formulations or if exposure to them lasts for several days, the urine samples should be taken in the morning following the cessation of exposure. There are no generally accepted biological exposure indices established for dithiocarbamates (Liesivuori and Savolainen, 1994).
There are presently no widely accepted methods for biological monitoring of exposure to dithiocarbamates, mainly due to the lack of knowledge of their pharmacokinetics in humans, and the lack of adequate analytical chemistry methods. Therefore, the measurement of urinary carbon disulfide (CS_2) by thiothiazolidine-4-carboxylic acid (TTCA), which is a metabolite of almost all dithiocarbamates, has been suggested as an index to assess high-level exposure to these compounds.
TTCA measurements in urine since 2011 have demonstrated no exceedances above the professional limit value of 5 mg/g cr (creatinine). The large majority of the samples (>95%) are below the ground signal of 1 mg/g cr, with a significant amount even below the detection limit.

2.6.9.2 Data collected on humans

Two articles were provided in this section: in the first of them, dated 1975, occurrence of occupational contact dermatitis after Vapam was reported in the agricultural and industrial workers from the district of Neubrandenburg (former Germany Democratic Republic). It is stated that new cases have no longer occurred due to targeted occupational safety regulations. The second article is about the metam chemostasis (through the TRPA1 channel system) and lachrymator effects; it does not impact the hazard or risk assessment and can be used as supporting information.

2.6.9.3 Direct observation

About fifteen papers were provided in this section: generally, they report on known effects consecutive to exposure to metam: (allergic) contact dermatitis, eye irritation, upper respiratory problems, vomiting,…notably observed as a consequence of the pesticide spill near Dunsmuir (California, 1991). In extreme cases, metam poisoning (by ingestion) may end up in coma and death in humans (example of a suicide, after which autopsy revealed necrotic mucosa in the oesophagus, stomach, and proximal part of the duodenum). The importance of establishing, training workers on, and enforcing safety protocols in occupational settings and ensuring that workers are provided with appropriate personal protective equipment is highlighted.

Most data in the section are in accordance with the hazards identified in the dossier and can be used as supplemental data for the weight of evidence assessment.

2.6.9.4 Epidemiological studies

In this section, most data are in accordance with the hazards identified in the dossier and most can be used as supporting information. It is, however, suggested that, in spite of limited resources and by using medical records and questionnaires, valuable human toxicology data (for instance odor perception...) can be obtained from epidemiological studies of accidental human exposures.

2.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

Based on data from animal studies with toxic doses, the signs of toxicity are likely to be lachrymation, blepharospasm, conjunctivitis and chemical burns after contact with eyes, primary irritation, dermatitis, eczema, chemical burns after contact with skin, nausea, vomiting, burns, gastroenteritis, sleepiness, cardiac and respiratory disorders, shock, renal lesions after ingestion, and primary mucosal and upper respiratory tract irritation, throat pain, cough hoarseness after inhalation.

There are no generally accepted biological exposure indices established for dithiocarbamates (Liesivuori and Savolainen, Toxicology Jun 17;91(1):37-42. 1994).
There are presently no widely accepted methods for biological monitoring of exposure to dithiocarbamates, mainly due to the lack of knowledge of their pharmacokinetics in humans, and the lack of adequate analytical chemistry methods. Therefore, the measurement of urinary carbon disulfide (CS_2) by thiothiazolidine-4-carboxylic acid (TTCA), which is a metabolite of almost all dithiocarbamates, has been suggested as an index to assess high-level exposure to these compounds.

Medical surveillance in the manufacturing plant relies on the following:
- Specific attention is paid to complaints: skin irritation, allergic reactions of skin and lungs, others.
- Blood testing: RBC, Hb, Hct, MCV, MCH, WBC and formula, GOT, GTP, g-GT, AF, creatinine, TSH, T4.
- Urine testing:TTCA, thiothiazolidine-4-carboxylic acid, Zn Mn.. Elimination (excretion of metabolites) happens in 2 phases in the urine: after 2-6h and 65-70h.
- Tests for lung function: Vital capacity and Forced, Expiratory Volume in one second.

2.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

If inhaled: Move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Treat symptomatically. If symptoms persist, call a physician.
In case of skin contact: Wash off with soap and plenty of water. Wash off immediately with plenty of water for at least 15 minutes. Wash contaminated clothing before re-use. In the case of skin irritation or allergic reactions see a physician.
In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. If easy to do, remove contact lens, if worn. Call a physician or poison control center immediately.
If swallowed: Seek medical advice. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person.
Medical treatment: this compound is considered harmful if swallowed and if inhaled. Metam sodium belongs to the group of dithiocarbamates. There is no specific antidote. Supportive care should be given and the medical treatment should be based on the judgment of the physician in response to the reaction of the patient.

2.6.9.7 Expected effects of poisoning

The prognosis for the expected effects of acute exposure through inhalation of metam sodium and MITC is that it results in respiratory irritation, eye irritation and signs of gastrointestinal complaints.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 66: Overview of relevant studies for derivation of reference values for risk assessment

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Oral 8-day drinking water study (pilot), mouse (C57/BL/10JfAP/Alpk) 0, 0.35, 0.62 mg/mL (10-15/sex/dose) No guideline followed (RF study) Not GLP compliant, supplemental	**Metam sodium** B. n°: Y06930/007 Purity: 525.54 g/L	0.35 mg/mL n.a.	0.62 mg/mL n.a.	↓body weight, ↓water consumption, slight ↓food consumption (younger ♀)
Oral 21-day drinking water study (pilot), rat (Alpk:APfSD-Wistar derived) 0, 0.1, 0.3, 0.7 mg/mL (5/sex/dose) Similar to OECD 407 (1981) (RF study) Not GLP compliant, supplemental	**Metam sodium** B.n°: 11877-9-1 Purity: 32.8 g/L	<0.1 mg/mL n.a.	0.1 mg/mL n.a.	↓bodyweight, ↓water and food consumption, ↑relative kidney weight and ↓absolute spleen weight
Oral 90-day drinking water study, mouse (C57BL/10JfAP/Alpk) 0, 0.018, 0.088, 0.35, 0.62 mg/mL (15/sex/dose) Actual intake (mg/kg bw/d) °: 0, 0.79, 4.48, 36, 60 (♂) 0, 1.05, 5.82, 38, 64 (♀) Similar to OECD 408 (2018) GLP compliant, acceptable	**Metam sodium** B.n°: BAS/005/00 N Purity: 525.54 g/L	0.018 mg/mL **= 0.8 mg/kg bw/d**	0.088 mg/mL = 4.5 mg/kg bw/d	haematological findings (↓Hb, Hct and RBC count) and histopathological changes, apparent in the urinary bladder (eosinophilic granules in transitional epithelial cells) *At doses > 0.088 mg/mL*: ↓body weight and body weight gain, ↓food and water consumption, ↑relative liver weight, pale liver showing accentuated lobular pattern and mucosal hyperplasia of urinary bladder.
Oral 90-day drinking water study, rat (Alpk:APfSD-Wistar derived) 0, 0.018, 0.089 and 0.443 mg/mL (12/sex/dose) Actual intake (mg/kg bw/d) ° 0, 0.49, 3.10, 18.51 (♂) 0, 0.73, 3.68, 21.05 (♀) U.S. EPA 82-1, Similar to OECD 408 (2018) GLP compliant, acceptable	**Metam sodium** B. n° BAS/005/00 N Purity: 525.54 g/L	0.018 mg/mL **= 0.5 mg/kg bw/d**	0.088 mg/mL = 3.1 mg/kg bw/d	↓body weight, ↓body weight gain, ↓food and water consumption ↓urinary volume, ↑specific gravity *At doses > 0.088 mg/mL*: altered clinical chemistry and urinalysis, overt histopathologic alterations in the olfactory epithelium.
Oral (gavage) 90-day study, dog (Beagle) 0, 1, 5 and 10 mg/kg bw/d (4♂,4♀) U.S. EPA 82-1 Not fully in agreement with OECD 409 (1998-1981) GLP compliant, acceptable	**Metam sodium** B. n° BAS/005/00 N Purity: 43.15%	**1 mg/kg bw/d**	5 mg/kg bw/d	↑clinical signs (salivation, food regurgitation), altered haematological and clinical chemistry parameters, ↑urinary bladder cell mitosis, hepatitis, biliary inflammatory cell infiltration.

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Oral 12-13 weeks study, + recovery after 8 weeks, dog (Beagle) 10 mg/kg bw/d (1♂,1♀, no control) No guideline followed Not GLP compliant, supplemental (limited to liver injury, investigation on reversibility)	**Metam sodium** B. n°: BAS/005/00 N Purity: 43.14%	n.a.	n.a.	↑plasma ALT in weeks 10-12 ♀ and 11-13 ♂ ↑pigmented macrophages / Kupffer cells. No macroscopic findings or effects on liver weight. Observed effects considered reversible.
Oral (gavage) 1-year study, dog (Beagle) 0, 0.05, 0.1 or 1 mg/kg bw/d (4♂,4♀) U.S. EPA 83-1 Not fully in agreement with OECD 452 (1981). GLP compliant, acceptable	**Metam sodium** B. n° BAS/005/00 N 90-2 Purity : 43.148%	**0.1 mg/kg bw/d**	1 mg/kg bw/d	Blood chemistry effects (↑kaolin-cephalin time, ↑ALT/ASP/ALP, ↑liver histopathological findings (hepatocyte and Kupffer-cell pigmentation).
Inhalation 65-day study, rat (CD -Sprague Dawley) measured concentration: 0, 6.5, 45, 160 mg/m3 (18♂,18♀) Duration: 6h/d, 5d/week Corresponding to 0, 1.75, 12.1, 42.6 mg/kg bw/d metam 0, 0.19, 0.59, 1.54 mg/kg bw/d MITC Equivalent to OECD 413 (1981) GLP compliant, acceptable	**Vapam technical (metam sodium)** B. n° EHC 0355-21; Purity: 32.7%.	(LOCAL) <6.5 mg/m^3 < **1.75 mg/kg bw/d** (SYSTEMIC) 6.5 mg/m^3 =**1.75 mg/kg bw/d**	6.5 mg/m^3 =**1.75 mg/kg bw/d** 45 mg/m^3 = **12.1 mg/kg bw/d**	Histopathology nasal passage:↑lymphocytic rhinitis, ↑hyperplasia of mucogenic epithelium (top-dose ↑lung histiocytosis, ↑mucigenic cyst) ↓b.w. gain, ↓food consumption, ↑ALP, ↑liver mononuclear cell infiltration. *Top-dose local/systemic effects* (160 mg/m³): Liver: ↑rel. weight and ↓albumin (liver damage), Stomach: ↑glandular duct ectasia, erosive/ ulcerative gastritis, urinary tract transitional epithelium hyperplasia/ metaplasia, Kidney: ↑relative weight, ↑pyelitis), Adrenals, Lungs: ↑relative weight.
Inhalation 21-day study, rat (Sprague-Dawley) 0, 0.51, 1.54, 4.53 mg/L (25♂,25♀) Not in agreement with OECD guideline 413(1981) Not GLP compliant, supplemental (not accepted): dosage duration and administration not clearly reported, MMAD not reported, too high droplet size, respiration fraction uncertain.	**Metam sodium** B. n°: not reported Purity: 42.4%	**0.51 mg/L** n.a.	1.54 mg/L n.a.	↓Body and ↓organ weights, ↑Hb, lung histopathology: ↑alveolar emphysema, ↑bronchopneumonic foci, bronchial epithelial metaplasia, inflammation. *Top-dose findings*: ↑foam cell pneumonia, atelectasis, foam cell foci, bronchiectasis, ↑thymus atrophy, changes in urinary bladder, inflammatory nasal cavity, ↓spermatogenesis, prostate atrophy.
Percutaneous 21-day study, rabbit (White Russian) 0, 31.25, 62.5 or 125 mg/kg bw/d (5♂,5♀ intact skin, 5♂,5♀ abraded skin) Duration: 8h/d, 21d	**Metam sodium** B. n°: BAS-00500-N Purity: 42.4%	LOCAL: **31.25 mg/kg bw/d** SYSTEMIC: **125 mg/kg bw/d**	62.5 mg/kg bw/d >125 mg/kg bw/d	erythema, oedema, rhagades, epidermodermatitis No systemic toxicity.

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Equivalent to OECD 410 (1981) Not GLP compliant, acceptable				
2 year, drinking water LT toxicity /carcinogenicity study, rat (Hsd/Ola: Wistar-Tox) Doses tested: 0, 0.019, 0.056, 0.19 mg/mL = 0, 1.5, 4.3,12.5 mg/kg bw/d (♂) 0, 2.7, 6.8, 16.8 mg/kg bw/d (♀) Equivalent to OECD 453 (1981) GLP compliant	**Metam sodium** Batch n° BAS/005/OON 90-2 Purity: 525.54 g/L = 43.148% w/w	Systemic: **1.5 mg/kg bw/d**	Systemic: 4.3	↓body weight (♀), ↓food (♀) and ↓water consumption, haematological and urinalysis changes (♂,♀). ↑non-neoplastic lesions in the liver (spongiosis/peliosis) (♂,♀), adrenal vascular ectasy (♂), Harderian gland mononuclear cell infiltration (♂), Steno's gland atrophy (♂,♀) or adenitis (♀), rhinitis (♀), Bowman's gland /duct hypertrophy (♂), spleen porphyria (♂) and uterus glandular dilatation. *Top-dose*: ↑olfactory and respiratory epithelial hyperplasia (♂,♀), spleen haemosiderosis (♂,♀) and myopathy (♂).
		Carcino: **1.5 mg/kg bw/d**	Carcino: 4.3	Considering animals surviving >1 year: trend-like ↑incidence of haemangioma + haemangiosarcoma (♂). Top-dose ↑ hepatocellular adenocarcinoma, and 1 hepatoblastoma (♂). Conclusion: **Carc 2, H351 «Suspected of causing cancer»**
2 year, drinking water LT toxicity /carcinogenicity study, mouse (C57BL/10JfCD-1/Alpk) Doses tested: 0, 0.019, 0.074, 0.23 mg/mL bw/d. = 0, 1.9, 7.2 , 28.9 mg/kg bw/d (♂) 0, 2.6, 9.6 and 31.2 mg/kg bw/d (♀) Equivalent to OECD 451 (1981) GLP compliant	**Metam sodium** Batch n° BAS/005/00N 90-2 Purity: 525.54 g/L (43.148%)	Systemic: **<1.9 mg/kg bw/d**	Systemic: 1.9	↑eosinophilic inclusion bodies in urinary bladder epithelial cells (♂,♀). *At 7.2 mg/kg bw/d and/or above:* ↓body weight (♂), food (♂) and water consumption (♂,♀), ↑liver w (♂,♀), ↑kidney weight (♀). ↑non-neoplastic urinary bladder lesions: ↑epithelial hyperplasia (♂,♀), ↑submucosal hyalinisation, ↑connective tissue at 7.2 mg/kg b.w./d (♀) and above (♂,♀). Liver toxicity: ↑vacuolation (♂,♀) at the top-dose and hepatocellular necrosis (♀) at 7.2 mg/kg b.w./d and above. *Top-dose*: ↑urinary bladder submucosal inflammatory cell infiltration (♂,♀).
		Carcino: **<1.9 mg/kg bw/d**	Carcino: 1.9	↑angiosarcoma at any site (♂). *At 7.2 mg/kg bw/d and/or above:* ↑angiosarcoma, in the spleen (♂), in the liver (♀) and at any site (♂,♀). *Top-dose*: ↑transitional cell papilloma/carcinoma of the urinary bladder Conclusion: Carc 2, H351 «Suspected of causing cancer»

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Two-generation drinking water, rat (Alpk:APfSD - Wistar-derived) 0, 0.01, 0.03 and 0.10 mg/mL = 0, 1.4, 4.0 and 12.3 mg/kg bw/d (♂) 0, 2.0, 4.9 and 14.4 mg/kg bw/d (♀) Equivalent to OECD 416 (1983) - GLP compliant	**Metam sodium** Batch n° BAS/005/00 N Purity: 526 g/L = 43.148% w/w	Parental **4.0 mg/kg bw/d**	Parental 12.3	↓body weight during premating, gestation and lactation. Olfactory gland duct: ↑dilatation and hypertrophy with alveolar cell loss and olfactory epithelium degeneration; lymphocytic infiltration in nasolacrimal duct.
		Offspring: **4.0 mg/kg bw/d**	Offspring: 12.3	↓pup and litter weight
		Repro: **12.3 mg/kg bw/d**	Repro: >12.3	-
Oral (gavage) developmental study, rat (Chbb, THOM- Wistar-derived, SPF) 0, 10, 40, 120 mg/kg bw/d. 25 animals/dose; Day 6-15 of gestation Equivalent to OECD 414 (1981) - GLP compliant	**Metam sodium** Batch n° ZH 130585 Purity: 517.3 g/L (42.20%)	Maternal: **10 mg/kg bw/d**	Maternal: 40	↓body weight (gain) and food consumption
		Developm.: **<10 mg/kg bw/d**	Developm.: 10	↑dose-related incidence of skeletal variations and retardations (ossification delays). *≥40 mg/kg/d:* ↓foetal weight gain *Top-dose*: 2/1 meningocoele, 1 bilateral microphthalmia.
Oral (gavage) developmental study, rat (Alpk:APfSD- Wistar-derived, SPF) – range finding study 0, 20, 40, 80 mg/kg bw/d 10 animals/dose; Day 7-16 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	**Metam sodium** Batch n° BAS/005/00 N Purity: 526 g/L = 43.148% w/w	Maternal: **<20 mg/kg bw/d**	Maternal: 20	↓body weight gain and food consumption.
		Developm.: **<20 mg/kg bw/d**	Developm.: 20	↓foetal weight, ↑post-implantation losses, intra-uterine deaths *Top-dose:* 1 meningocoele
Oral (gavage) developmental study, rat (Alpk:APfSD (Wistar-derived, SPF) – full study 0, 5, 20, 60 mg/kg bw/d 24 animals/dose; Day 7-16 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	**Metam sodium** Batch n° BAS/005/00 N – 90/2 Purity: 526 g/L = 43.148% w/w	Maternal: **5 mg/kg bw/d**	Maternal: 20	↓body weight (gain), ↓food consumption, salivation, stain around mouth, urinary incontinence, vaginal bleeding, kidney pelvic dilatation.
		Developm.: **<5 mg/kg bw/d**	Developm.: 5	↑unossified odontoid, cervical vertebrae and calcaneum (variants); a minor defect in a cervical vertebrae center. *≥20 mg/kg/d:* ↓foetal weight, ↑several vertebral column ossification delays. *Top-dose:* ↑post-implantation losses, intra-uterine deaths, 1 unossified cervical arches, 2/2 microphthalmia, 1 anophthalmia, 1 short upper jaw/cleft lip, 3/3 internal hydrocephaly, 1 cerebral meningocoele, ↑*manus/pes* scores (↓ossification).
Oral (gavage) developmental study, rabbit (NZW) – range-finding studies	**Metam sodium**	Maternal: **5 mg/kg bw/d**	Maternal: 20	↓faecal output, ↓body weight gain, ↓food consumption *Top-dose:* ↑sloughed mucosa glandular stomach.

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
0, 5, 20, 40, 60 mg/kg bw/d 8 animals/dose; Day 8-20 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	Batch n° BAS/005/00 N – 90/2 Purity: 526 g/L = 43.15% w/w	Developm.: **20 mg/kg bw/d**	Developm.: 40	↓foetal weight, ↑post-implantation losses, intra-uterine deaths. *Top-dose:* 1 *cyclopia*
Oral (gavage) developmental study, rabbit (NZW) – full study 0, 5, 20, 60 mg/kg bw/d 24 animals/dose; Day 8-20 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	**Metam sodium** Batch n° BAS/005/00 N – 90/2 Purity: 525.54 g/L = 43.14% w/w	Maternal: **5 mg/kg bw/d**	Maternal: 20	↓body weight gain, ↓food consumption
		Developm.: **5 mg/kg bw/d**	Developm.: 20	↑minor skeletal defects, ↓ossification *Top-dose:* ↓foetal weight, ↑post-implantation losses, intra-uterine deaths, 1 cerebral meningocoele, 2/2 cleft palate
Oral (gavage) developmental study, rabbit (Himalayan Chbb:HM- outbred) – full study 0, 10, 30, 100 mg/kg bw/d 24 animals/dose; Day 6-18 of gestation. Equivalent to OECD 414 (1981) - GLP compliant	**Metam sodium** Batch n° ZH 130585 Purity:517.3 g/L = 42.2% w/w	Maternal: **10 mg/kg bw/d**	Maternal: 30	↓body weight gain
		Developm.: **<10 mg/kg bw/d**	Developm.: 10	↑post-implantation loss, intra-uterine deaths, number of dead implants, ↑visceral variations *≥30 mg/kg/d:* gall bladder agenesis, asymetrical *sternebrae*. *Top-dose:* ↑*truncus arteriosus communis*, 1 *spina bifida*, 1 meningocoele
Oral acute (gavage) study, rat, (Crl:CD® BR) 0, °50, 750, °1500 mg/kg bw/d (12-16/sex/dose) No guideline followed GLP compliant, supplemental	**Metam sodium** Batch n° not reported Purity: 43.15%	Systemic: **50 mg/kg bw**	Systemic: 750 mg/kg bw	↓body weight, ↓water consumption, slight ↓food consumption (younger ♀) *Top-dose*: ↑mortality.
		Neurotoxic: **<50 mg/kg bw**	Neurotoxic: 50 mg/kg b.w	↓(loco)motor activity *≥750 mg/kg bw* alterations in posture and palpebral closure; lachrimation, salivation, fur appearance, respiratory rate); alterations in arousal and gait, ↑time to 1st step, ↓rearing activity); absent approach, olfactory and pupil responses, absent tail pinch response, ↓tail pinch stimulus reaction time, ↓startle response; ↓hindlimb extensor strength).
Oral 90-day drinking water study, rat (Alpk:APf SD-Wistar derived) 0, 0.02, 0.06 and 0.20 mg/mL (12/sex/dose) Actual intake (mg/kg bw/d) ° 0, 2.0, 6.0, 14.7 (♂) 0, 3.3, 8.4, 17.8 (♀) Comparable to OECD 424 (1997) GLP compliant, acceptable	**Metam sodium** Batch n°: BAS/005/00 N 90-2 Purity : 43.148%	Systemic: **<2 mg/kg bw/d**	Systemic: 2 mg/kg bw/d	↓body weight ↓food consumption
		Neurotoxic: **14.7 mg/kg bw/d**	Neurotoxic: >14.7 mg/kg bw/d	No signs of neurotoxicity

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
MITC				
28-day inhalation study (pilot), rat (Wistar) , 5/sex/dose 0, 1.7, 6.8, 34 ppm 0, 5, 20, 100 mg/m^3 0, 1.35, 5.4, 27.0 mg/kg b.w./d Similar to OECD 412 (1981) (RF) GLP compliant.	**MITC** B. n°: 205MK Purity: 96.9%	Local: <5 mg/m^3 **<1.35 mg/kg bw/d**	Local: 5 mg/m^3 1.35 mg/kg bw/d	Nasal cavity: ↑focal atrophy of olfactory epithelium.
		Systemic: 5 mg/m^3 **1.35 mg/kg bw/d**	Systemic: 20 mg/m^3 5.4 mg/kg bw/d	↓body weight (gain), ↑clinical signs, ↑non-focal atrophy olfactory epithelium, ↑neutrophils. *At top dose:* ↑bilirubin and thromboplastin time in ♂, ↑lung weight.
28-day inhalation range-finding study, rat (Crl:CD SD), (5/sex/dose) 0, 5, 20, 40, 80 ppm 0, 15, 60, 119, 235 mg/m3 0, 4.21, 16.15, 32.05, 63.21 mg/kg b.w./d Similar to OECD 412 (1981) (RF) GLP compliant	**MITC** B. n°: 56198PJV Purity: 99.7%	Local: 15 mg/m^3 **4.21 mg/kg bw/d**	Local: 60 mg/m^3 16.15 mg/kg bw/d	↑histopathological alterations in the respiratory tract.
		Systemic: <15 mg/m^3 **<4.21 mg/kg bw/d**	Systemic: 15 mg/m^3 4.21 mg/kg bw/d	↑clinical signs, ↓body weight gain (>10%), ↓thymus weight. *At top-dose (235 mg/m³, 63 mg/kg b.w./d):* ↑mortality
28-day inhalation range-finding study, mouse (♂,♀CD-1 and ♀B6C3F1) (5/sex/dose) 0, 5, 20, 40, 80 ppm 0, 15, 60, 119, 235 mg/m3 0, 4.21, 16.15, 32.05, 63.21 mg/kg b.w./d Similar to OECD 412 (1981) (RF) GLP compliant	**MITC** B. n°: 56198PJV Purity: 99.7%	Local: <15 mg/m^3 **<4.0 mg/kg bw/d**	Local: 15 mg/m^3 4.0 mg/kg bw/d	↑histopathological alterations in the respiratory tract (nasal squamous epithelium hyperplasia)
		Systemic: <15 mg/m^3 **<4.0 mg/kg bw/d**	Systemic: 15 mg/m^3 4.0 mg/kg bw/d	CD-1: ↑clinical signs, ↓body weight gain (>20%), ↓thymus weight.
28-day inhalation mechanistic study, rat (Crl:CD SD), (8♂/dose) 0, 0.5, 5, 20 ppm 0, 1.5, 15, 60 mg/m3 0, 0.4, 4.0, 16 mg/kg b.w./d Similar to OECD 412 (1981) (mechanistic study); GLP compliant	**MITC** B. n°: 56198PJV Purity: 99.7%	Local: 1.5 mg/m^3 **0.4 mg/kg bw/d**	Local: 15 mg/m^3 4.0 mg/kg bw/d	↑histopathological alterations in the respiratory tract, ↑DNA replication (BrdU labelling).
		Systemic: 1.5 mg/m^3 **0.4 mg/kg bw/d**	Systemic: 15 mg/m^3 4.0 mg/kg bw/d	↓body weight gain (>10%)
90-day oral (gavage) study, dog (Beagle) 0, 0.04, 0.4 or 2 mg/kg bw/d (4/sex/dose) OECD 409 (1998) GLP compliant	**MITC** B. n° BX 340178/ AD 11308/ BX 340178 AD 11328 Purity: 95.96%	**0.4 mg/kg bw/d**	2 mg/kg bw/d	↓body weight, haematological findings, single blood chemistry parameters, ↑thymic involution, liver coarse texture and ↑severity of lipid periportal depots and vacuolation.
90-day whole-body inhalation study, rat (Crl:CD SD) (10/sex/dose) 0, 1, 5, 15 ppm 0, 3, 15, 45 mg/m3 0, 0.81, 4.0, 12 mg/kg b.w./d OECD 413 (1981) GLP compliant	**MITC** B. n°: 56198PJV Purity: 99.7%	Local: 3 mg/m^3 **0.81 mg/kg bw/d**	Local: 15 mg/m^3 4.0 mg/kg bw/d	↑histopathological alterations in the respiratory tract
		Systemic: 15 mg/m^3 **4.0 mg/kg bw/d**	Systemic: 45 mg/m^3 12 mg/kg bw/d	haematology and coagulation parameters (↑APTT, ↑neutrophils), ↑liver and ↓thymus weight

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
90-day whole-body inhalation study, mouse (CD-1) (10/sex/dose) 0, 1, 5, 20 ppm 0, 3, 15, 60 mg/m3 0, 0.88, 4.4, 13.2 mg/kg b.w./d OECD 413 (1981) GLP compliant	**MITC** B. n°: 56198PJV Purity: 99.7%	Local: 3 mg/m^3 **0.88 mg/kg bw/d**	Local: 15 mg/m^3 4.4 mg/kg bw/d	↑histopathological alterations in the respiratory tract
		Systemic: 15 mg/m^3 **4.4 mg/kg bw/d**	Systemic: 60 mg/m^3 13.2 mg/kg bw/d	↓body weight (gain), ↓food consumption, ↑clinical signs, haematological (↓WBC and ↑neutrophil counts) and clinical-chemical (↑bilirubin), ↓spleen and ↓thymus weights, and ↑microscopic findings in the nasal cavity
2-year oral (drinking water) toxicity and carcinogenicity study, rat (Crl:CD (SD)). Doses tested: 0, 2, 10, 50 ppm 0, 0.08, 0.44, 1.60 mg/kg bw/d (♂) 0, 0.12, 0.66 and 2.65 mg/kg bw/d for (♀) OECD 453 (2018) Non GLP compliant	**MITC** B.n°: 28 166; 29 482; Purity: 95.36% - 96.06%	Systemic **0.44 mg/kg bw/d**	Systemic 1.60 mg/kg bw/d	↓Body weight, altered WBC parameters, bone marrow hyperplasia, ↑kidney microcalculi, liver effects, and spleen hyperplasia/ ↑haematopoiesis
		Carcinogenicity **1.60 mg/kg bw/d**	Carcinogenicity >1.60 mg/kg bw/d	No treatment-related ↑tumour incidence observed
2-year inhalation chronic toxicity and carcinogenicity study, rat (Crl:CD(SD)) Doses tested: 0, 0.5, 5, 20 ppm 0, 1.5, 15, 60 mg/m³ 0, 0.65, 6.5, 26 mg/kg b.w./d OECD 453 (2018) GLP compliant	**MITC** B. n°: 56198PJV; Purity: 97.2% - 99.7%	Local <0.5 ppm <1.5 mg/m³ **<0.65 mg/kg bw/d**	Local 5 ppm 1.5 mg/m³ 0.65 mg/kg bw/d	↑squamous metaplasia , ↑olfactory nasal epithelium degeneration, ↑epithelial hyperplasia, ↑squamous metaplasia on the larynx. *At higher doses*, ↑eyes opacity,bilateral keratitis and various adverse findings in nasal tissues, larynx, trachea, lungs, olfactory bulbs.
		Systemic 5 ppm 15 mg/m³ **6.5 mg/kg bw/d**	Systemic 20 ppm 60 mg/m³ 26 mg/kg bw/d	No ↑mortality vs. control incidence, but at top-dose death caused by nasal tumours. Other relevant effects: ↓body weight (>20%), ↓body weight gain, ↑haematology/clinical chemistry findings.
		Carcinogenicity 5 ppm 15 mg/m³ **6.5 mg/kg bw/d**	Carcinogenicity 20 ppm 60 mg/m³ 26 mg/kg bw/d	↑malignant and benign nasal tumours, 1 benign papilloma in the lung Possible MoA: corrosive action of MITC, no in-vivo genotoxicant, but human relevance not excluded. Conclusion: carcinogenic in the rat after administration of MITC via inhalation. **Carc 2, H351 «Suspected of causing cancer»**
2-year chronic oral (drinking water) toxicity and carcinogenicity study, Mouse (ICR:JCL)	**MITC**, B. n° MS 25206; Purity: 93.14%	Systemic 20 ppm **3.3 mg/kg bw/d**	Systemic 80 ppm 12 mg/kg bw/d	↑Clinical signs, ↓body weight, ↓body weight gain, slight effects in blood, and altered organ weights.

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Doses tested: 0, 5, 20, 80, 200 ppm 0, 0.82, 3.3, 11.83, 25.71 mg/kg bw/d (♂) 0, 0.91, 3.66, 13.03, 29.03 mg/kg bw/d (♀) OECD 453 (2018) Non GLP compliant		Carcinogenicity 200 ppm **25 mg/kg bw/d**	Carcinogenicity >200 ppm >25 mg/kg bw/d	No treatment-related ↑tumour incidence observed Not carcinogenic in the mouse after oral administration of MITC.
18-month inhalation carcinogenicity study, Mouse (Crl:CD-1) Doses tested: 0, 1, 5, 15 ppm 0, 3, 15, 45 mg/m³ 0, 1.35, 6.7, 20 mg/kg b.w./d OECD 453 (2018) GLP compliant	**MITC,** B. n°: 56198PJV; Purity: 97.2% - 99.7%	Local <1 ppm <3 mg/m³ **<1.35 mg/kg bw/d**	Local 1 ppm 3 mg/m³ 1.35 mg/kg bw/d	↑respiratory and transitional hyperplasia of nasal epithelium; Dose-dependent ↑nasal findings (incidence and severity, minimal to mild)
		Systemic 1 ppm 3 mg/m³ **1.35 mg/kg bw/d**	Systemic 5 ppm 15 mg/m³ 6.7 mg/kg bw/d	↓body weight, ↓body weight gain, ↓spleen weights (♂)
		Carcinogenicity 15 ppm 45 mg/m³ **20 mg/kg bw/d**	Carcinogenicity >15 ppm 45 mg/m³ 20 mg/kg bw/d	Neoplastic finding Single incidence of (benign) papilloma, insufficient for classification. Not carcinogenic in the mouse after administration of MITC via inhalation.
Oral (drinking water) two-generation reproductive toxicity study, SD- rat Dose levels: 0, 2, 10, or 50 ppm 0, 0.16. 0.74, 3.49 mg/kg bw/d (♂) 0, 0.20, 0.94, 4.49 mg/kg bw/d(♀) OECD 416 (1983) – GLP compliant	**MITC**, B.n°: 340178; 95.86 – 96.51%	Parental: 10 ppm **0.71 mg/kg bw/d**	Parental: 50 ppm 3.6 mg/kg bw/d	↓body weight gain
		Offspring: 50 ppm **3.6 mg/kg bw/d**	Offspring: >50 ppm >3.6 mg/kg bw/d	No treatment-related effect *Note: the relevance of 2 litters with 2 pups showing hydrocephaly at the 2 highest doses should be further documented by means of HCD.*
		Reproductive: 50 ppm **3.6 mg/kg bw/d**	Reproductive: >50 ppm >3.6 mg/kg bw/d	No treatment-related effect
Inhalation two-generation reproductive toxicity study, SD- rat – full study Dose levels: 0, 1, 5, 20 ppm 0, 3, 15, 60 mg/m3 0, 0.8, 4.0, 16 mg/kg bw/d OECD 416 (2001) – GLP compliant	**MITC,** B. n°: 56198PJV; 99.7%	Parental: 5 ppm 15 mg/m³ **4.0 mg/kg bw/d**	Parental: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↑clinical signs, ↓body weight (gain), ↓food consumption and efficiency, ↑adrenal w., ↓thymus w.; lung: ↑epithelial regeneration, fibrosis, inflammation, ulceration (F_0,F_1); brain: ↑olfactory bulb atrophy* (F_0,F_1) *(*dose w/o effect yet undetermined)*
		Offspring: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	Offspring: >20 ppm >60 mg/m³ >16 mg/kg bw/d	No treatment-related effect
		Reproductive: 5 ppm 15 mg/m³ **4.0 mg/kg bw/d**	Reproductive: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↑oestrous cycle length (F_0,F_1); ↑gestation length (F_0,F_1); ↑vaginal patency delay (F_1); ↑primordial follicle counts (F_1)* *(*dose w/o effect yet undetermined)*
Inhalation one-generation reproductive toxicity study, SD- rat – range-finding study	**MITC,** B. n°: 56198PJV; 99.7%	Parental: 10 ppm 30 mg/m³ **8.0 mg/kg bw/d**	Parental: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↓body weight (gain), ↓food consumption

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Dose levels: 0, 5, 10, 20 ppm 0, 15, 30, 60 mg/m3 0, 4.05, 8.0, 16 mg/kg bw/d OECD 421 (1995) – GLP compliant		Offspring: 10 ppm 30 mg/m³ **8.0 mg/kg bw/d**	Offspring: 20 ppm 60 mg/m³ 16 mg/kg bw/d	↓body weight (gain) during pre-weaning period (F_1).
		Reproductive: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	Reproductive: >20 ppm >60 mg/m³ >16 mg/kg bw/d	No treatment-related effect
Oral (gavage) developmental toxicity study, Wistar rat, from GD 6–15 Dose levels: 0, 3, 10, 30 mg/kg bw/d OECD 414 (1981) – GLP compliant	**MITC,** B. n° ZH 6205 MK, 96.9%	Maternal: **3 mg/kg bw/d**	Maternal: 10 mg/kg bw/d	↓body weight gain, ↑water consumption
		Developmental: **10 mg/kg bw/d**	Developmental: 30 mg/kg bw/d	↓foetal weight, ↓placental weight, ↑runts No adverse developmental findings.
Inhalation developmental toxicity study, SD- rat, from GD 6–20 Dose levels: 0, 1, 4, 12 ppm, 0, 3.12, 12.39, 36.33 mg/m³ 0, 0.8, 3.2, 9.7 mg/kg bw/d OECD 414 (2001) – GLP compliant Dose levels identified in a rat RF study at 0, 1, 5, 20 ppm; ↓b.w., f.c., foetal w.) at 20 ppm.	**MITC,** B. n°: 56198PJV, 99.7%	Maternal: 4 ppm 12 mg/m³ **3.2 mg/kg bw/d**	Maternal: 12 ppm 36 mg/m³ 9.7 mg/kg bw/d	↓body weight and body weight gain; ↓food consumption
		Developmental: 4 ppm 12 mg/m³ **3.2 mg/kg bw/d**	Developmental: 12 ppm 36 mg/m³ 9.7 mg/kg bw/d	↑major blood vessel variation; ↓ossification of the 13th rib
Oral (gavage) developmental toxicity study, Chinchilla- rabbit, from GD 6 – 18 Dose levels: 0, 1, 3, 10 mg/kg bw/d, OECD 414 (1981) – GLP compliant	**MITC**, B. n° ZNT 85/231-2; 98%	Maternal: **3 mg/kg bw/d**	Maternal: 10 mg/kg bw/d	↓body weight and body weight gain
		Developmental: **10 mg/kg bw/d**	Developmental: >10 mg/kg bw/d	No adverse developmental findings.
Inhalation developmental toxicity study, NZW- rabbits, from GD 7 – 28 Dose levels: 0, 1, 5, 15 ppm 0, 3, 15, 45 mg/m³ 0, 0.2, 1.1, 3.5 mg/kg bw/d OECD 414 (2001) – GLP compliant Dose levels identified in (i) inhalational RF study at 0, 1, 4, 12 ppm; ↓b.w., ↓f.c., ↓foetal w) at 12 ppm and in (ii) a 21d- pilot study at 0, 5, 10, 20, 60 ppm, fatalities at ≥20 ppm, and clinical signs, ↓b.w.c., f.c. at ≥5 ppm.	**MITC**, B. n°: 56198PJV; 99.7%	Maternal: 5 ppm 15 mg/m³ **1.1 mg/kg bw/d**	Maternal: 15 ppm 45 mg/m³ 3.5 mg/kg bw/d	*At beginning of dosage*:↑clinical signs, ↑mortality, ↑moribundity, ↓body weight. *Further*: ↓body weight, ↓food consumption, ↑maternal macroscopic findings
		Developmental: 5 ppm 15 mg/m³ **1.1 mg/kg bw/d**	Developmental: 15 ppm 45 mg/m³ 3.5 mg/kg bw/d	Very slightly altered litter parameters: ↑♂/♀ ratio, ↓foetal viability, ↑early resorption, ↑post implantation loss; single developmental findings (omphalocoele, vertebral centra anomaly, small spleen, 7th sternebra, irregular ossification of 6th sternebra.

Study	Test substance Batch n°, Purity	NOAEL	LOAEL	Critical effect
Inhalation acute neurotoxicity study, SD-rat Dose levels : 0, 20, 40, 80 ppm 0, 60, 120, 240 mg/m³ 0, 16, 32, 65 mg/kg bw/d OECD 424 (1997) GLP compliant The selected doses are acceptable, with the top-dose (240 mg/m³) being ~40% LC50 (540 mg/m³).	**MITC** Batch n° 51198PJV, Purity 99.7%	Local <20 ppm <60 mg/m³ **<16 mg/kg bw/d**	Local: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	↑olfactory epithelium necrosis, ↑transitional epithelium degeneration on d1.
		Systemic: <20 ppm <60 mg/m³ **<16 mg/kg bw/d**	Systemic: 20 ppm 60 mg/m³ **16 mg/kg bw/d**	↓absolute and relative liver weight *At ≥40 ppm:* ↑clinical signs; *At 80 ppm:* ↓b.w. (change).
		Neurotoxic: 40 ppm 120 mg/m³ **32 mg/kg bw/d**	Neurotoxic: 80 ppm 240 mg/m³ **65 mg/kg bw/d**	↑axonal degeneration, possibly associated with hindlimb functionality (d15). Note: NT observations confounded by systemic toxicity: *At ≥20 ppm:* ↓rotarod performance, ↑eyelids completely and/or half-closed; *At ≥40 ppm:* ↑slightly soiled fur appearance, ↑oral crusty deposits, ↓rearing , ↑grooming activity; ↓locomotor activity (d0), unremarkable or less prominent at d7 and totally resorbed at d14.
28-Day inhalation immunotoxicity study, ♀mouse (B6C3F1) Dose levels : 0, 1, 3, 10 ppm 0, 3, 9, 30 mg/m³ 0, 1.4, 4, 13 mg/kg bw/d OECD 424 (1997) GLP compliant **RMS** : in the view of minimal toxicity achieved at the top-dose, and the limited number of immunological parameters investigated, this study is considered to provide complementary information, only.	**MITC** Batch n° 51198PJV, Purity 99.7%	Systemic: 10 ppm 30 mg/m³ **13 mg/kg bw/d**	Systemic: >10 ppm >30 mg/m³ **>13 mg/kg bw/d**	No adverse effect
		Immunotoxicity: 10 ppm 30 mg/m³ **13 mg/kg bw/d**	Immunotoxicity: >10 ppm >30 mg/m³ **>13 mg/kg bw/d**	No adverse effect

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

Metam ADI

During the former evaluation, the ADI was set to **0.001 mg/kg bw/d**, based on the 1-year dog drinking water study (NOAEL = 0.1 mg/kg bw/d, safety factor = 100). As the metam study package has not been modified since, the same value is proposed again by the RMS.

$ADI_{metam\ sodium}$ = 0.001 mg/kg bw/d

MITC ADI

The ADI proposed by the RMS (supported by the notifier) is **0.004 mg/kg bw/d**, based on the 90-day dog study drinking water study (NOAEL = 0.4 mg/kg bw/d, safety factor = 100), in accordance with the former evaluation.

ADI_{MITC} = 0.004 mg/kg bw/d

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure – ARfD (acute reference dose)

Metam ARfD

During the former evaluation, it was decided to set ARfD to **0.1 mg/kg bw/d**, based on overall rat developmental study and supported by rabbit developmental study (NOAEL = 10, safety factor = 100). The metam study package has not been modified since and the same value is proposed again by the RMS.

$ARfD_{metam\ sodium}$ = 0.1 mg/kg bw/d

MITC ARfD

The ARfD proposed by the **RMS** is **0.03 mg/kg bw/day** based on the NOAEL for rat maternal toxicity (at 3 mg/kg bw/day in both the rat and the rabbit developmental study, oral route) with a safety factor=100, in accordance with the former evaluation in 2011.

$ARfD_{MITC}$ = 0.03 mg/kg bw/d

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

Metam AOEL

During the former evaluation, it was decided to set ARfD to **0.001 mg/kg bw/d**, based on the 1-year drinking water study in dogs (NOAEL = 0.1 mg/kg bw/d, safety factor = 100). The metam study package has not been modified since and the same value is proposed again by the RMS.

$AOEL_{metam\ sodium}$ = 0.001 mg/kg bw/d

MITC AOEL

The AOEL proposed by the **RMS** is **0.004 mg/kg bw/d**, based on the 90-day dog drinking water study (NOAEL = 0.4 mg/kg bw/d, safety factor = 100), in accordance with the former evaluation in 2011.

$AOEL_{MITC}$ = 0.004 mg/kg bw/d

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

Metam AAOEL

During the former evaluation, no metam AAOEL was derived. The RMS proposes to set ARfD to **0.1 mg/kg bw/d**, based on overall rat developmental study and supported by rabbit developmental study (NOAEL = 10, safety factor = 100).

$AAOEL_{metam\ sodium}$ = 0.1 mg/kg bw/d

MITC AAOEL

During the former evaluation, no metam AAOEL was derived. The RMS proposes to set the AAOEL to 0.0037 (rounded to **0.004 mg/kg bw/d**), based on the inhalation rabbit developmental study (maternal NOAEL =1.1 mg/kg bw/d, safety factor 300 = 100 × 3). The NOAEL is based upon ↑clinical signs, ↑mortality, ↑moribundity at beginning of dosage. The additional safety factor of 3 takes into account the severity of the effect at LOAEL (lethality).

$AAOEL_{MITC}$ = 0.004 mg/kg bw/d

2.6.11 Summary of product exposure and risk assessment

2.6.11.1 Taminco product - Metam Na 510 SL (volume 20)

Product information and toxicological reference values for MITC

Product name and code	Metam Na 510 SL (Metam K 690 SL)
Formulation type	SL
Category	Soil fumigant
Active substance	Metam-sodium 510 g/L (Metam-potassium 690 g/L)
AOEL systemic	0.004 mg/kg bw/d)
AAOEL systemic	0.004 mg/kg bw/d)
Inhalation absorption	100%
Oral absorption	100%
Dermal absorption	This route of exposure is not relevant for exposure to MITC

Application of Metam Sodium 51% SL is performed by by indoor drip irrigation in greenhouses

Summary of use pattern, application rates and equipment

Crop treated (before planting)	Application rate of Metam Na 510 SL	Application rate of metam-sodium	Method
Lettuce	300 L/ha (250)*	153 kg a.s./ha *(172)	Drip irrigation with gas tight (totally impermeable) TIF film (6 weeks) in permanent structures
Ornamentals	300 L/ha (250)*	153 kg a.s./ha *(172)	
Baby leaf	150-200 L/ha (125-167)*	77-102 kg a.s./ha *(86-115)	

* Application rate expressed for the equivalent potassium salt product 'Metam K 690 SL'

For the DRAR renewal, Taminco generated indoor drip irrigation exposure studies under the conditions of the new GAP. These indoor, drip-irrigation studies were conducted at the lower application rate which is now the supported GAP. These new data also incorporated the use of totally impermeable film (TIF), which, from the time of application, will remain in place for 6 weeks before removal.

These studies for which operator, bystanders & residents, and worker data were provided are:

- A greenhouse spring study performed in Spain ([redacted], 2020)
  Operator data: **CP7.2.1/08**
  Bystanders and residents data: **CP7.2.2/09**
  Worker data: **CP7.2.3/07**

- A greenhouse spring study performed in Belgium ([redacted] 2020a))
  Operator data: **CP7.2.1/09**
  Bystanders and residents data: **CP7.2.2/10**
  Worker data: **CP7.2.3/08**

- A greenhouse autumn study performed in Spain ([redacted] 2020b)[$]
  Operator data: **CP7.2.1/10**
  Bystanders and residents data: **CP7.2.2/11**
  Worker data: **CP7.2.3/09**

$: of note, this study erroneously used 108 kg a.s/ha instead of 153 kg a.s/ha.

The notable results from these studies are reported in **Part I**

In addition, in what regards bystanders and residents, air dispersion modelling studies using measured air concentrations of MITC from the three monitoring studies (see above) were performed to estimate MITC emissions:

An air dispersion modelling for residential and bystander risk assessment accompanying the Spanish spring trial performed in 2019 (Exponent Inc., 2020a):
CP7.2.2/14

An air dispersion modelling for residential and bystander risk assessment accompanying the Belgian spring trial performed in 2019 (Exponent Inc., 2020b):
CP7.2.2/15

An air dispersion modelling for residential and bystander risk assessment accompanying the Spanish autumn trial performed in 2019 (Exponent Inc., 2020c):
CP7.2.2/16

The notable results from these air monitoring studies are reported in **Part II**

Part I

Operator

Studies CP7.2.1/08, CP7.2.1/09, CP7.2.1/10

Summary of operator (fumigator) exposure and comparison with AOEL/AAOEL for MITC: Maximum air concentration measurements for studies performed in Spain and Belgium – representative data for GAP

Person monitored	**Air concentration of MITC (µg/m³)**	**In the absence of RPE**		**In the presence of RPE§**	
		***Systemic exposure (mg/kg bw/d)**	**% of current AOEL (0.004 mg/kg bw/day)**	***Systemic exposure (mg/kg bw/d)**	**% of current AOEL (0.004 mg/kg bw/day)**
Poly tunnel performed in Spain ([redacted], 2020, CP7.2.1/08)					
Operator 1	15.44	0.0026	64	0.00013	3.2
Operator 2	11.78	0,002	49	0.0001	2.5
Greenhouse study performed in Belgium ([redacted], 2020a, CP7.2.1/09)					
Operator 1	58.7	0.01	245	4.9×10^{-4}	1.2
Operator 2	1.52 (based on LOD)	0.0003	6	1.3×10^{-5}	0.3
Operator 3	1.42 (based on LOD)	0.0002	6	1.2×10^{-4}	0.3
Greenhouse study performed in Spain ([redacted], 2020b, CP7.2.1/10)					
Operator 1	3.21	0.0005	13	2.7×10^{-5}	0.7
Operator 2	3.56	0.0006	15	3.0×10^{-5}	0.7

Calculations assume a respiration rate of 1.25 m³/h for moderate activity and a default body weight of 60 kg for operators.

*Exposure is calculated assuming an 8 hour working day.

§: Respiratory protective equipment: mask with organic vapour filter (respirator with A1P2-filter) allowing 5% penetration.

Operator - Conclusions:
[redacted], 2020: levels of exposure for operators are within the currently established AOEL if RPE are worn.

[redacted] 2020a: levels of exposure for operators are within the currently established AOEL if RPE are worn.

[redacted], 2020b: levels of exposure for operators are within the currently established AOEL if RPE are worn.

Thus, the new exposure study data, which are representative of the supported GAP, confirm that levels of exposure for operators (fumigators) are within the currently established AOEL of 0.004 mg/day bw/day if RPE are worn.

Bystanders/residents

Studies CP7.2.2/09, CP7.2.2/10, CP7.2.2/11

Summary of bystander risk assessment for MITC – Studies representative of supported GAP

Distance from structure (and height from ground	Maximum Air concentration of MITC (µg/m3)	% of current AOEL(0.004 mg/kg bw/day)	Risk Management Measures required
[redacted] 2020, CP7.2.2/09 study performed in Spain (spring use) - Maximum air concentration (worst case)			
5 m (1.5 m)	10.93	10.9	None
5 m (0.5 m)	7.26	34.5	
50 m (1.5 m)	6.565	6.6	None
50 m (0.5 m)	7.825	37.2	
[redacted] 2020a, CP7.2.2/10 study performed in Belgium (autumn use) - Maximum air concentration (worst case)			
5 m (1.5 m)	4.19	4.2	None
5 m - 20 m (0.5 m)	*4.34	20.6	
[redacted] 2020b, CP7.2.2/11 study performed in Spain (autumn use) - Maximum air concentration (worst case)			
5 m (1.5 m)	19.15	19	None
5 m (0.5 m)	39.74	**189**	See below
15 m (1.5 m)	8.66	8.7	Bystanders should be restricted to **a minimum distance of 15 m** from the treated structure for 48 hours from the time the fumigation is initiated
15 m (0.5 m)	11.54	55	

Risk assessment assumes a 1 hour exposure for bystanders and EFSA guidance hourly breathing rates of 0.04 m3/hour/kg for a 60 kg adult and 0.19 m3/hour/kg for a 10 kg child < 3 years old.

*Highest air concentration measured at the 0.5m sampling height was measured at 50m distance during sampling interval S11.

Bystander - Conclusions:
[redacted], 2020: levels of exposure for bystanders are within the currently established AOEL of 0.004 mg/day.

[redacted], 2020a: levels of exposure for bystanders are within the currently established AOEL of 0.004 mg/day

[redacted], 2020b: bystanders should be restricted to a **minimum distance of 15 m** from the treated structure for 48 hours from the time the fumigation is initiated.

Thus, from the new exposure study data, which are representative of the supported GAP, it may be concluded that bystanders should be restricted to a **minimum distance of 15 m** from the treated structure for 48 hours from the time the fumigation is initiated.

Summary of resident risk assessment for MITC – Studies representative of supported GAP

Distance from structure (and height from ground	Air concentration of MITC (µg/m3)	% of current AOEL(0.004 mg/kg bw/day)	Risk Management Measures required
[redacted] **2020, CP7.2.2/09 study performed in Spain (spring use) - Maximum 24 hour TWA air concentration (worst case)**			
5 m(1.5 m)	2.93	16.8	None
5 m (0.5 m)	2.076	74	
[redacted] **2020a, CP7.2.2/10 study performed in Belgium (autumn use) - Maximum 24 hour TWA air concentration (worst case)**			
5 m(1.5 m)	4.03	23	None
5 m (0.5 m)	3.54	94.7	
[redacted] **2020b, CP7.2.2/11 study performed in Spain (autumn use) - Maximum 24 hour TWA air concentration (worst case)**			
15 m (1.5 m)	6.65	33.8	None
15 m (0.5 m)	8.6	**230**	**See below**
50 m (1.5 m)	3.21	18.4	Applications should only b made **at a minimum distanc of 50 m** of dwellings which ma be occupied.
50 m (0.5 m)	3.1	83	

Risk assessment assumes a 24 hour exposure for residents and EFSA guidance daily breathing rates of 0.23 m^3/day/kg for a 60 kg adult and 1.07 m^3/day/kg for a 10 kg child < 3 years old.

Resident -Conclusion

[redacted], 2020: levels of exposure for residents are within the currently established AOEL of 0.004 mg/day.

[redacted], 2020a: levels of exposure for residents are within the currently established AOEL of 0.004 mg/day.

[redacted], 2020b: an acceptable risk assessment is achieved provided applications are made **a minimum distance of 50 m** of dwellings which may be occupied (AOEL 0.004 mg/kg bw/day).

Thus, from the new exposure study data, which are representative of the supported GAP, it may be concluded that an acceptable risk assessment is achieved provided applications are made a **minimum distance of 50 m** of dwellings which may be occupied (AOEL 0.004 mg/kg bw/day).

Worker

Summary of worker (re-entry) exposure and comparison with AOEL for MITC:
Exposure studies performed in Spain and Belgium in 2019 – Representative data for GAP

Person monitored	Trial phase	Air concentration of MITC (µg/m³)	In the absence of RPE		In the presence of RPE[c]	
			Systemic exposure (mg/kg bw/d)	% of current AOEL (0,004 mg/kg bw/d)	Systemic exposure (mg/kg bw/d)	% of current AOEL (0,004 mg/kg bw/d)
Study performed in Spain in spring ([redacted], 2020, CP7.2.2/09)						
Worker 1	Re-entry after application (DAT 42) for venting and removal of TIF	0.0143[a]	2.4×10^{-5}	0.6	1.2×10^{-6}	0.03
Worker 2		0.0143[a]	2.4×10^{-5}	0.6	1.2×10^{-6}	0.03
Worker 3		0.0143[a]	2.4×10^{-5}	0.6	1.2×10^{-6}	0.03
Worker 4		0.0143[a]	2.4×10^{-5}	0.6	1.2×10^{-6}	0.03
Study performed in Belgium in autumn ([redacted], 2020a, CP7.2.2/10)						
Worker 1	Re-entry after application (DAT 42) for venting and removal of TIF	3.35	0.0006	14	2.8×10^{-5}	0.7
Worker 2		4.4	0.0007	18	3.7×10^{-5}	0.9
Study performed in Spain in autumn ([redacted], 2020b, CP7.2.2/11)						
Worker 1	Re-entry after application (DAT 42) for venting and removal of TIF	0.381[b]	6.4×10^{-5}	1.6	3.2×10^{-6}	0.08
Worker 2		0.385[b]	6.4×10^{-5}	1.6	3.2×10^{-6}	0.08
Worker 3		0.398[b]	6.4×10^{-5}	1.6	3.2×10^{-6}	0.08
Worker 4		0.398[b]	6.4×10^{-5}	1.6	3.2×10^{-6}	0.08

Parameters for calculations assume a respiration rate of 1.25 m^3/h for moderate duty and a default body weight of 60 kg for workers, and a work duration of 8h/d.

[a]Measured values were below LOQ. Air concentration calculated assuming LOQ (0.03 µg/tube) x air sampling rate (2L/minute)
x duration of task (mins)

[b]Measured values were below LOD. Air concentration calculated assuming LOD (0.009 µg/tube) x air sampling rate (0.2L/minute) x duration of task (mins)

[c]Respiratory protective equipment: mask with organic vapour filter (respirator with A1P2-filter) allowing 5 % penetration.

Worker – Conclusions

[redacted] 2020: levels of exposure for workers are within the currently established AOEL of 0.004 mg/day bw/day if RPE are worn.

[redacted] 2020a: levels of exposure for workers are within the currently established AOEL of 0.004 mg/day bw/day if RPE are worn.

[redacted], 2020b: levels of exposure for workers are within the currently established AOEL of 0.004 mg/day bw/day if RPE are worn.

Thus, the new exposure study data, which are representative of the supported GAP, confirm that levels of exposure for re-entry workers are low and within the AOEL without the use of RPE.

Part II

Air dispersion modelling

Air dispersion modelling using measured air concentrations of MITC from three air monitoring studies ([redacted] 2020 **CP 7.2.2/09**, [redacted] 2020a **CP 7.2.2/10**, [redacted] 2020b **CP 7.2.2/11**) was performed to estimate MITC emissions from representative, commercially sized greenhouses, located in central and southern Europe. These air monitoring studies are reported in full at **CP 7.2.2/14**, **CP 7.2.2/15** and **CP 7.2.2/16**.

Summary of bystander risk assessment for MITC – Studies representative of supported GAP

	Distance	**95th percentile 1-hr average MITC concentration (µg/m³)**	**Risk Management Measured required**
[redacted] 2020, study performed in Spain (spring use), modelisation study CP 7.2.2/14			
Child	5	14.2	For an (A)AOEL of 0.004 mg/kg bw/day no buffer zone is required
Adult	5	12.3	
[redacted] 2020a, study performed in Belgium (autumn use), modelisation study CP 7.2.2/15			
Child	35	20.2	For an (A)AOEL of 0.004 mg/kg bw/day bystanders should be restricted to a minimum distance of **35 m** from the treated structure for 48 hours from the time the fumigation is initiated
Adult	35	16.2	
[redacted] 2020b, study performed in Spain (autumn use), nodelisation study CP 7.2.2/16			
Child	35	20.3	For an (A)AOEL of 0.004 mg/kg bw/day bystanders should be restricted to a minimum distance of **35 m** from the treated structure for 48 hours from the time the fumigation is initiated
Adult	35	16.7	

Bystanders - Conclusions

CP 7.2.2/14: air concentration levels of MITC for bystanders are within acceptable levels at the 5m distance.

CP 7.2.2/15: bystanders should be restricted to **a minimum 35m** from the greenhouse for 48 hours from the time the fumigation is initiated.

CP 7.2.2/16: bystanders should be restricted to **a minimum 35m** from the greenhouse for 48 hours from the time the fumigation is initiated.

Thus, from the air dispersion modelling studies, it may be concluded that bystanders should be restricted to a **minimum 35m** from the greenhouse for 48 hours from the time the fumigation is initiated.

Summary of resident risk assessment for MITC – Studies representative of supported GAP

	Distance	**75th percentile 24-hr average MITC concentration (µg/m³)**	**Risk Management Measured required**
[redacted] 2020, study performed in Spain (spring use),), modelisation study CP 7.2.2/14			
Child	5	4.4	For an (A)AOEL of 0.0112 mg/kg bw/day no buffer zone is required
Adult	5	3.7	
Child	15	3.2	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of 15 m of dwellings which may be
Adult	15	2.8	

			occupied.
[redacted] **2020a, study performed in Belgium (autumn use),), modelisation study CP 7.2.2/15**			
Child	5	6.1	For an (A)AOEL of 0.0112 mg/kg bw/day no buffer zone is required
Adult	5	4.8	
Child	30	3.4	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of 30 m of dwellings which may be occupied.
Adult	30	2.8	
[redacted] **2020b, study performed in Spain (autumn use),), modelisation study CP 7.2.2/16**			
Child	5	6.0	For an (A)AOEL of 0.0112 mg/kg bw/day no buffer zone is required
Adult	5	4.9	
Child	35	3.6	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of 35 m of dwellings which may be occupied.
Adult	35	3.0	

Summary of resident risk assessment for MITC – Studies representative of supported GAP

	Distance	**75th percentile 24-hr average MITC concentration (µg/m3)**	**Risk Management Measured required**
[redacted] **2020, study performed in Spain (spring use),), modelisation study CP 7.2.2/14**			
Child	15	3.2	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of **15 m** of dwellings which may be occupied.
Adult	15	2.8	
[redacted] **2020a, study performed in Belgium (autumn use),), modelisation study CP 7.2.2/15**			
Child	30	3.4	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of **30 m** of dwellings which may be occupied.
Adult	30	2.8	
[redacted] **2020b, study performed in Spain (autumn use),), modelisation study CP 7.2.2/16**			
Child	35	3.6	For an AOEL of 0.004 mg/kg bw/day applications should only be made at a minimum distance of **35 m** of dwellings which may be occupied.
Adult	35	3.0	

Residents - Conclusions

[redacted], 2020: for **resident** exposure, applications should not be made within **15m** of dwellings which may be occupied.

[redacted], 2020a: for **resident** exposure, applications should not be made within **30m** of dwellings which may be occupied.

[redacted], 2020b: for **resident** exposure, applications should not be made within **35m** of dwellings which may be occupied.

Thus, from the air dispersion modelling studies, it may be concluded that for residents, applications **should not be made within 35m** of dwellings which may be occupied.

Overall summary - Taminco Metam Na 510 SL (Metam K 690 SL).

Summary of risk assessment for operators, workers, residents and bystanders for Metam Na 510 SL (Metam K 690 SL)

	Result	PPE / Risk mitigation measures (indoor drip irrigation)
Operators	Acceptable	As MITC is toxic via ingestion, toxic via inhalation, skin corrosive and skin sensitiser, RPE/PPE are necessary including respiratory mask (A1P2), (oral)mask, protective gloves, and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product.
Bystanders	Acceptable	- Indoor drip irrigation: * A buffer zone of minimum 35 m should be applied as the minimum distance from treated plots.
Residents	Acceptable	- Indoor drip irrigation: * A buffer zone of minimum 50 m should be applied as the minimum distance between treated plots and residential and public buildings.
Workers	Acceptable	As MITC is toxic via ingestion, toxic via inhalation, skin corrosive and skin sensitiser, RPE/PPE are necessary including mask (A1P2), (oral)mask, protective gloves, and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product.

2.6.11.2 Lainco product - Metam Sodium 51% SL (volume 21)

Product information and toxicological reference values for MITC

Product name and code	Metam Sodium 51% SL
Formulation type	Soluble Liquid (SL)
Category	Soil fumigant
Active substance	Metam sodium 510 g/L
AOEL systemic	0.004 mg/kg bw/d) (MITC)
Inhalation absorption	100%
Oral absorption	100%
Dermal absorption	Not applicable

Application of Metam Sodium 51% SL is performed by a soil-injection technique using tractor-mounted equipment as well as by indoor drip irrigation in greenhouses.

For the DRAR renewal, no recent data were provided by Lainco. This notifier presented an exposure study from [redacted] (2014, **CP7.2.1.2/01**), obtained before the former GAP.

Three MITC usages were examined:

- **Soil injection**: 153 kg a.s./ha, soil sealed;
- **Indoor drip irrigation**: 612 kg a.s./ha, polyethylene low density plastic sheets (thickness 30 µm) (thus not with TIF).
- Outdoor drip irrigation, which is no longer supported

In addition, in the exposure study, workers were involved in drilling holes after 18 ± 1 days of the product application, although the former GAP indicates a re-entry period of 21 days for greenhouses:
"*After 18 ± 1 days of the product application, the greenhouse doors and windows were opened for 5-10 minutes and then each worker drilled holes on the plastic sheets using a tool specifically designed for this purpose for approximately 30 minutes (depending on the greenhouse size)*."

In the Lainco dossier, it was stated :"*In order to provide additional support to a safe use for Metam Sodium 51% SL, new data are being generated with the use of TIF to cover the treated area. The first results will be available in 2020*". However, such data obtained with TIF were not received by the RMS.

Operator

Study **CP7.2.1.2/01** ([redacted], 2014)

Soil injection

Table 2.6-1 Operator exposure statistical analyses and comparisons to AOEL

	Residue in filter (ng)	Residue (mg/m^3)	Exposure (mg/kg bw/day)			% AOEL		
			No RPE	RPE (90% protection)	RPE (98% protection)	No RPE	RPE (90% protection)	RPE (98% protection)
Breathing zone								
75th perc Empirical	23533	0.0474	0.00691	0.000691	0.000138	173%	**17%**	**3.5%**
75th perc Theoretical	45053	0.0903	0.0132	0.00132	0.000263	330%	**33%**	**6.6%**
95th perc Empirical	300242	0.606	0.0884	0.00884	0.00177	2210%	221%	**44%**
95th perc Theoretical	370991	0.748	0.109	0.0109	0.00218	2725%	273%	**55%**
Inside cab								
75th perc Empirical	24033	0.0483	0.00704	0.000704	0.000141	176%	**18%**	**3.5%**
75th perc Theoretical	46936	0.0944	0.0138	0.00138	0.000275	345%	**35%**	**6.9%**
95th perc Empirical	328602	0.668	0.0973	0.00973	0.00195	2434%	243%	**49%**
95th perc Theoretical	393975	0.798	0.116	0.0116	0.00233	2900%	290%	**58%**
Outside cab								
75th perc Empirical	79723	0.160	0.0234	0.00234	0.000467	584%	**58%**	**12%**

	Residue in filter (ng)	Residue (mg/m^3)	Exposure (mg/kg bw/day)			% AOEL		
			No RPE	RPE (90% protection)	RPE (98% protection)	No RPE	RPE (90% protection)	RPE (98% protection)
75th perc Theoretical	129723	0.261	0.0381	0.00381	0.000761	953%	**95%**	**19%**
95th perc Empirical	998740	2.04	0.298	0.0298	0.00595	7441%	744%	149%
95th perc Theoretical	1707448	3.47	0.505	0.0505	0.0101	12625%	1263%	253%

Conclusion for operator risk assessment during (outdoor) soil injection
A safe use is not expected for the operator applying Metam Sodium 51% SL by soil injection without respiratory protective equipment (RPE).
The operator exposure to MITC during soil injection application of Metam Sodium 51% SL becomes acceptable when RPE (FFP2 or A1P2) is worn. This is in agreement with the MITC classification as toxic via inhalation. Under a conservative approach, i.e. when considering the 95th percentiles, a **RPE A1P2 would be requested**.
The residue levels measured in the breathing zone of the operators and inside the cabin are similar. The residue levels measured outside the cabin cannot be considered directly for operator exposure but lead to the confirmation that a tractor equipped with a cabin is mandatory for an application by soil injection.
As MITC is also toxic via ingestion, skin corrosive and skin sensitiser, **PPE are also necessary including protective gloves, mask and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product**.

Indoor drip irrigation

Table 2.6-2 Operator exposure statistical analyses and comparisons to AOEL

	Residue in filter (ng)	Residue (mg/m^3)	Exposure (mg/kg bw/day)	% AOEL
			No RPE	No RPE
75th perc Empirical	558	0.00107	0.000157	**3.9%**
75th perc Theoretical	236	0.000443	0.000266	**6.7%**
95th perc Empirical	3326	0.00660	0.000962	**24%**
95th perc Theoretical	1587	0.00304	0.00198	**50%**

Conclusion for operator risk assessment during indoor drip irrigation
A safe use is expected for the unprotected operator applying Metam Sodium 51% SL by drip irrigation under indoor conditions, even based on data generated at twice the dose rate (612 kg a.s./ha instead of 306 kg a.s./ha) and with a conservative approach (considering the 95th percentiles), as the highest percentage of AOEL is 50%.
MITC is a severe respiratory irritant, and as such respiratory protection such as a half mask with either FFP2 filter or combination filter A1P2 should be worn during the whole process. A respiratory protection factor of 90% or 98% is afforded by this type of RPE, respectively.
As MITC is also toxic via ingestion, skin corrosive and skin sensitiser, PPE are also necessary including protective gloves, mask and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product.

Bystander/resident

Study **CP7.2.1.2/01** ([redacted], 2014)

Soil injection

Table 2.6-3 Bystander exposure statistical analyses and comparisons to AOEL

	Exposure (mg/kg bw/day)		% AOEL	
	Child	**Adult**	**Child**	**Adult**
0 m				
95th perc - Empirical	0.0874	0.0184	2186%	460%
95th perc - Theoretical	0.191	0.0402	4775%	1005%
5 m				
95th perc - Empirical	0.0831	0.0175	2077%	437%
95th perc - Theoretical	0.184	0.0388	4600%	970%
15 m				
95th perc - Empirical	0.0809	0.0170	2022%	426%
95th perc - Theoretical	0.265	0.0557	6625%	1393%
25 m				
95th perc - Empirical	0.0856	0.0180	2141%	451%
95th perc - Theoretical	0.292	0.0614	7300%	1535%

Table 2.6-4 Resident exposure statistical analyses and comparisons to AOEL

	Exposure (mg/kg bw/day)		% AOEL	
	Child	**Adult**	**Child**	**Adult**
0 m				
75th perc - Empirical	0.301	0.0647	7522%	1617%
75th perc - Theoretical	0.211	0.04540	5275%	1135%
5 m				
75th perc - Empirical	0.186	0.0400	4656%	1001%
75th perc - Theoretical	0.167	0.0359	4175%	898%
15 m				
75th perc - Empirical	0.221	0.0475	5524%	1187%
75th perc - Theoretical	0.193	0.0414	4825%	1035%
25 m				
75th perc - Empirical	0.206	0.0443	5152%	1107%
75th perc - Theoretical	0.170	0.0365	4250%	913%

The AOEL for bystander (child and adult) and resident (child and adult) was exceeded according to the statistical calculations done on the basis of the results of the [redacted] (2014) study. **A safe use is not expected** for those populations after soil injection application of Metam Sodium 51% SL and soil sealing, without further mitigation measures.

In addition to the product specific study ([redacted], 2014), a series of data from public domain was evaluated and considered in the DAR for metam sodium. A brief summary of these was presented in the FRR-Dec 2017. Further details were provided in the DAR August 2007, with revised data in the DAR July 2008. The following conclusions were agreed at EU level:

- Based on Saeed I.AM. et al., 2000: after application of Metam Sodium 51% SL by soil injection/chemigation, MITC measured in air 6 hours after outdoor application was 5.89 µg/m^3 that corresponds to an exposure of:
  - 0.00112 mg/kg bw/day (28% of AOEL) for a child bystander,
  - 0.000236 mg/kg bw/day (5.9% of AOEL) for an adult bystander,
  - 0.00630 mg/kg bw/day (158% of AOEL) for a child resident, and
  - 0.00135 mg/kg bw/day (34% of AOEL) for an adult resident.
- Based on Van de Berg F., 1993: after Metam Sodium 51% SL application by soil injection, MITC levels measured 1.1-9 d after application at 1.5 m height was 14 µg/m^3 that corresponds to an exposure of:
  - 0.00266 mg/kg bw/day (67% of AOEL) for a child bystander,
  - 0.000560 mg/kg bw/day (14% of AOEL) for an adult bystander,
  - 0.0150 mg/kg bw/day (375% of AOEL) for a child resident, and
  - 0.00322 mg/kg bw/day (81% of AOEL) for an adult resident.

Those data from the literature show that a safe use is possible for bystanders and residents, except for child residents. In the FRR released in December 2017, the zRMS considered the contamination of blank controls that were measured at sites where operators #3, #5 and #8 were monitored. Those contaminations happened at ≥150 m from the treated field. The highest residue level (1126 ng) represents 60% of the AOEL for a child resident.

Conclusion for bystander/resident risk assessment during (outdoor) soil injection

Using the measured data from the [redacted] (2014) study, bystander and resident exposure levels associated with soil injection of Metam Sodium 51% SL **do not show a safe use when soil is sealed** (no plastic sheet over the soil after application). This conclusion results from statistical calculations done considering the parameters and percentiles recommended in the EFSA Guidance Document [EFSA Journal 2014;12(10):3874] which are conservative.

The following risk mitigation measures are proposed to achieve a safe use of Metam Sodium 51% SL for bystanders and residents:

- A Totally Impermeable Film (TIF) should be applied immediately after soil injection application (instead of soil sealing).
- A buffer zone of **≥ 150 m** should be applied as the minimum distance between treated plots and residential and public buildings.

Other extra precautionary mitigation measures should be implemented:

- Resident(s) should be warned before and during a planned treatment.
- Safety cordon tape should be used to delimit the buffer zone.

Indoor drip irrigation

Table 2.6-5 Bystander exposure statistical analyses and comparisons to AOEL

	Exposure (mg/kg bw/day)		% AOEL	
	Child	**Adult**	**Child**	**Adult**
0 m				
95th perc - Empirical	0.0447	0.00940	1117%	235%
95th perc - Theoretical	0.106	0.0222	2650%	555%
5 m				
95th perc - Empirical	0.0102	0.00216	256%	**54%**
95th perc - Theoretical	0.0709	0.0149	1773%	373%
15 m				
95th perc - Empirical	0.00433	0.000912	108%	**23%**
95th perc - Theoretical	0.0355	0.00747	888%	187%

25 m				
95th perc - Empirical	0.00238	0.000501	**59%**	**13%**
95th perc - Theoretical	0.0186	0.00392	465%	**98%**

Table 2.6-6 Resident exposure statistical analyses and comparisons to AOEL

	Exposure (mg/kg bw/day)		**% AOEL**	
	Child	**Adult**	**Child**	**Adult**
0 m				
75th perc - Empirical	0.145	0.0312	3630%	780%
75th perc - Theoretical	0.132	0.0284	3300%	710%
5 m				
75th perc - Empirical	0.0319	0.00686	798%	172%
75th perc - Theoretical	0.0331	0.00711	828%	178%
15 m				
75th perc - Empirical	0.00927	0.00199	232%	**50%**
75th perc - Theoretical	0.0115	0.00247	288%	**62%**
25 m				
75th perc - Empirical	0.00399	0.000859	100%	**21%**
75th perc - Theoretical	0.00506	0.00109	127%	**27%**

According to the highest of the empirical/theoretical 95th percentiles calculated from the results of the [redacted] (2014) study, a safe use is expected for adult bystanders at a distance **≥ 25m**. **No safe use** is identified for child bystanders.
According to the highest of the empirical/theoretical 75th percentiles calculated from the results of the [redacted] (2014) study, a safe use is expected for adult residents at a distance **≥ 15m** after indoor drip irrigation application of Metam Sodium 51% SL**. No safe use** is identified for child residents.
In the [redacted] (2014) study, the doors/roof/walls of the greenhouses were closed during and after application and the soil had been covered with a plastic sheet before start of application.

In the FRR released in December 2017, the zRMS considered the contamination of blank controls that were measured at sites where operators #15 and #19 were monitored. Those contaminations happened at **≥ 120 m** from the treated greenhouse. The highest residue level (645 ng) represents 34% of the AOEL for a child resident.

Conclusion for bystander/resident risk assessment during indoor drip irrigation
Using the measured data from the [redacted] (2014) study, bystander and resident exposure levels associated with indoor drip irrigation of Metam Sodium 51% SL do not show a safe use for child residents. This conclusion results from statistical calculations done considering the parameters and percentiles recommended in the EFSA Guidance Document [EFSA Journal 2014;12(10):3874] which are conservative. In addition, the dose rate used in the [redacted] (2014) study was twice the dose rate proposed in the table of GAPs in this dossier.
The following risk mitigation measures are proposed to allow achieving a safe use of Metam Sodium 51% SL for bystanders and residents:

- A Totally Impermeable Film (TIF) should be used to cover the soil before the start of application [instead of a polyethylene low density plastic film which was used in the study].
- A buffer zone of **≥ 120 m** should be applied as the minimum distance between treated greenhouse and residential and public buildings.

Other extra precautionary mitigation measures should be implemented:

- Resident(s) should be warned before and during a planned treatment.
- Safety cordon tape should be used to delimit the buffer zone.

Worker

Study **CP7.2.1.2/01** ([redacted], 2014)

Soil injection

Considered covered by covered by data presented for this activity conducted after indoor drip irrigation (see below).

Indoor drip irrigation

Table 2.6-7 Worker exposure statistical analyses and comparisons to AOEL

	Residue in filter (ng)	Residue (mg/m^3)	Exposure (mg/kg bw/day)			% AOEL		
			No RPE	RPE (90% protection)	RPE (98% protection)	No RPE	RPE (90% protection)	RPE (98% protection)
Breathing zone								
75th perc Empirical	112	0.00125	0.000209	0.0000209	0.00000418	**5.2%**	**0.52%**	**0.10%**
75th perc Theoretical	400	0.00495	0.000825	0.0000825	0.00001650	**21%**	**2.1%**	**0.41%**
95th perc Empirical	6628	0.0769	0.0128	0.00128	0.000256	320%	**32%**	**6.4%**
95th perc Theoretical	7453	0.0800	0.0133	0.00133	0.0002670	333%	**33%**	**6.7%**

Conclusion for worker risk assessment during indoor drip irrigation

It can be concluded that worker re-entry (to drill holes on the plastic sheets) under a greenhouse after 18 ± 1 days of the drip irrigation application of Metam Sodium 51% SL is acceptable for the unprotected worker when considering the 75th percentiles.
However, given the classification of MITC as toxic *via* inhalation and ingestion, skin corrosive and skin sensitiser, PPE and RPE (gloves, mask and coveralls) should be worn at all times when re-entering an area treated with Metam Sodium 51% SL. A half mask with either FFP2 filter or combination filter A1P2 is recommended during re-entry. A respiratory protection factor of 90% or 98% is afforded by this type of RPE, respectively.
A minimum re-entry period of 21 days is covered by the [redacted] (2014) study data which were generated 18±1 days after application.
This conclusion applies also to workers re-entering a field treated either by soil injection or drip irrigation with the condition that the field is covered by a totally impermeable film for 21 days following application.

Lainco Metam Sodium 51% SL. Overall summary

Summary of risk assessment for operators, workers, residents and bystanders for Metam Sodium 51% SL

	Result	PPE / Risk mitigation measures
Operators	Acceptable	Soil injection, drip irrigation: As MITC is toxic via ingestion, toxic via inhalation, skin corrosive and skin sensitiser, RPE/PPE are necessary including respiratory mask (FFP2, A1P2), (oral)mask, protective gloves, and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product.
Bystanders	Acceptable	- Soil injection: * A buffer zone of minimum **150m** should be applied as the minimum

	Result	PPE / Risk mitigation measures
		distance from treated plots. - Indoor drip irrigation: * A buffer zone of minimum **120 m** should be applied as the minimum distance between treated plots and residential and public buildings.
Residents	Acceptable	Same as bystanders.
Workers	Acceptable	As MITC is toxic via ingestion, toxic via inhalation, skin corrosive and skin sensitiser, RPE/PPE are necessary including respiratory mask (FFP2, A1P2), (oral)mask, protective gloves, and coverall protective against chemical products, protective goggles, boots that must be worn at all times when handling and applying the product.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), storage stability of residues had not been investigated in a guideline-compliant study, but the stability of residues of MITC in trial samples upon storage had been discussed, given its high volatility. The need for frozen storage stability data on MITC was at that time waived on the basis of a statement referring to the fate and behaviour of MITC in the environment, physical/chemical properties of MITC and information from plant metabolism studies (in which no losses of volatile compounds were observed during the most critical stages of the sample work-up, i.e. thawing, homogenisation and extraction); cf. Vol.3 B.7.1 (Garcia de Oteyza, 2011). At that time, the case provided by the applicant (Taminco) was accepted on the basis that the plant metabolism data back up the zero residue situation (cf. EFSA, 2011). However, the assumption of a 'zero' residue situation has been invalided, due to quantifiable MITC residues found in crops (see 2.7.4) and hence, the case previously provided by the applicant has no longer been relied upon in the framework of renewal of a.s. approval.

In the framework of the application for renewal of approval, a new storage stability study with MITC and DMTU ([redacted], 2020) was provided by one of the two applicants (Lainco). Samples were homogenized and fortified with either MITC or DMTU. Another study investigating stability of MITC and DMTU in potatoes ([redacted], 2014) is available and had been previously submitted by Lainco at Member State level and in support of an MRL application (but was not accepted by the Evaluating Member State Spain)[4]. At request of the RMS, that study was resubmitted and reconsidered in the framework of renewal of approval for the sake of completeness. Finally, reference was made to an existing storage stability study ([redacted], 1998) with MITC and DMTU (on strawberries, tomatoes and peppers), which had previously been assessed in the framework of the EU peer review for dazomet, another MITC-generating active substance. A summary of the available data is presented here below.

With regard to **MITC**, the newest study results ([redacted], 2020) show a very quick and significant decline of residue concentration in the stored samples, within a few days. It is remarkable that the decline observed in the new study was considerably faster in comparison with the study previously assessed in the framework of the EU peer review of dazomet ([redacted], 1998); MITC was adequately recovered from tomatoes, peppers and strawberries for up to 4 weeks of frozen storage in the latter study. However, it was noted that also in the study by [redacted] (1998), poor recoveries of MITC (38-70%) were apparently encountered for 0-week and 2-weeks stored fortified samples when these had to be transferred to another (distillation) flask after fortification. Hence, although the reasons for the apparent discrepancy between guideline-compliant studies could not be identified, it is possible that instability of MITC residues is not merely dependent on temperature and storage duration, but also influenced to an important extent by sample preparation and handling.

With regard to **DMTU**: Although the study previously assessed in the framework of the EU peer review of dazomet ([redacted], 1998) indicated stability up to 12 months in tomatoes and peppers (high water content matrices) and strawberries (high acid content matrix), the new study ([redacted], 2020) revealed significant degradation of DMTU in some matrices (onion, cucumber, strawberries). In other matrices (lettuce, potato, carrot), adequate recoveries could only be obtained (up to the longest storage period of 6 months) if an anti-oxidant was added to the sample prior to fortification. Indeed, another study ([redacted], 2014) had also shown that DMTU spiked to homogenised potato samples (without addition of an anti-oxidant) had almost completely dissappeared after 1 month of frozen storage. Only from tomato, DMTU was still adequately recovered after the maximum storage period (181 days) without addition of anti-oxidant. The reason for the apparent discrepancy between guideline-compliant studies could not be identified.

[4] RMS notes that in 2014, Lainco submitted an MRL application to increase several EU MRLs (cf. EFSA-Q-2016-00117), but the proposed MRL amendments were rejected based on issues concerning storage stability and eventually, Lainco withdrew its MRL application (on 21/10/2020).

Category	Commodity	T (°C)	Stability period - MITC		Stability period - DMTU		Comment/Source
			Value	Unit	Value	Unit	
High water content	Onion	-18°C	**≤ 1**	days	**≤ 7**	days	New study (a)
	Tomato	-18°C	**≤ 3**	days	**181**	days	New study (a)
		-20°C	*≤ 4*	*weeks*	*12*	*months*	*Dazomet initial DAR (b)*
	Peppers	*-20°C*	*≤ 4*	*weeks*	*12*	*months*	*Dazomet initial DAR (b)*
	Cucumber	-18°C	**≤ 3**	days	**≤ 14**	days	New study (a)
	Lettuce	-18°C	**≤ 14**	days	**190***	days	New study (a)
High oil content	-						
High protein content	-						
High starch content	Potato	-18°C	**≤ 7**	days	**197****	days	New study (a)
		-20°C	*Insufficiently stable for 40 days*		*Insufficiently stable for 30 days*		*New study (c)*
	Carrot	-18°C	**≤ 3**	days	**190****	days	New study (a)
High acid content	Strawberry	*-20°C*	*≤ 4*	*weeks*	*12*	*months*	*Dazomet initial DAR (b)*
		-18°C	**≤ 1**	days	**≤ 30**	days	New study (a)
Processed products	-						
Others	-						

(a) [redacted], 2020 (new study submitted by Lainco for approval renewal – KCA 6.1/02)

(b) [redacted]*, 1998 (assessed in DAR dazomet – BE, 2009)*

(c) [redacted] *2014 (new study submitted by Lainco for approval renewal – KCA 6.1/01); no data for periods of storage < 1 month; a sharp decline for MITC and a very fast degradation for DMTU was observed.*

* Sodium sulphite (anti-oxidant) was added prior to sample homogenisation and spiking in order to prevent degradation/reaction (and thus improve recovery); without addition of the anti-oxidant, low recoveries were observed.

** L-cysteine hydrochloride monohydrate (anti-oxidant) was added prior to sample homogenisation and spiking in order to prevent degradation/reaction (and thus improve recovery); without addition of the anti-oxidant, low recoveries were observed.

Conclusions:

TAMINCO:

In the framework of renewal of approval, the following commodity categories and types[5] are relevant to the representative uses supported by Taminco:

- high water content:
  - leafy vegetables and fresh herbs (lettuce, baby leaf)

Note: The representative use on ornamentals is not relevant in this regard, as no residue trials were conducted on this non-edible crop.

Taminco has not provided any new data on storage stability in support of this application. Moreover, the previously peer reviewed studies (from dazomet EU dossier) concern another commodity type (fruiting vegetables/cucurbits: tomato and pepper) and the reliability of the results of this study have been challenged by the newly available study ([redacted], 2020) provided by the other applicant. The need for studies was waived by Taminco, with reference to the relatively short storage time of the samples from the new residue trials prior to analysis; analysis of both lettuce and baby leaf samples occurred as soon as possible after sampling and generally within 48 h of sampling with few exceptions; only for two lettuce samples (trials FR01 and IT08), the time between sampling and extraction of DMTU was more than 48 hours (max. 70 hours). The stability of MITC and DMTU in extracts (stored at ambient temperature for up to 28 hours for MITC and at 10 ± 2°C for up to 19 hours for DMTU) was demonstrated by the corresponding procedural recoveries, which showed a mean recovery value between 70-110%.

RMS concludes that, on the basis of the information available, the results of the new supervised residue trials on lettuce and baby leaf crops (Taminco – KCA 6.3.1/01 and KCA 6.3.2/01) can be considered sufficiently reliable in terms of storage stability and sample storage duration and conditions.

[5] in line with Annex I of OECD guideline 506 on 'Stability of Pesticide Residues in Stored Commodities' (16 October 2007)

LAINCO:

In the framework of renewal of approval, the following commodity categories and types are relevant to the representative uses supported by Lainco:

- high water content:
  - fruiting vegetables/cucurbits (pepper)
  - bulb vegetables (bulb onion)

- high starch content:
  - starchy root crop (potato)
  - roots of root/tuber vegetable (carrot)

The stability of MITC and DMTU was tested in three diverse commodity types in the category of high water content commodities (bulb vegetables, fruiting vegetables/cucurbits and leafy vegetables) and in two diverse commodity types in the category of high starch content commodities and, moreover, stability was investigated in the specific crop commodities of interest related to the representative uses supported by Lainco (see above). Therefore, no further examination of storage stability would be required if samples were stored (prior to extraction for analysis) for a period not longer than the period for which stability has been demonstrated in each of the studies conducted with the relevant matrix.

- However, on the basis of this data package and taking into account the variability in results observed between presumably similar matrices, extrapolation of results to other crops belonging to these (or other) commodity categories and types cannot be recommended for the time being.
- As the reasons for the apparent discrepancy between results from different, guideline-compliant studies for one and the same matrix (e.g. tomatoes) could not be elucidated, RMS considers it appropriate to consider only the lowest maximum stability period for judging the reliability of residue trial results (e.g. max. 3 days for MITC analysis and max. 181 days for DMTU analysis in tomatoes).
- As regards **peppers**: taking into account the fast decline observed for MITC in other high water content matrices, also the 4 weeks/12 months (MITC/DMTU) stability period derived from the older study can be questioned. As pepper was not re-investigated in a second study, the stability of MITC and DMTU in this matrix is affected by an additional uncertainty. Looking at all the results for the commodity type fruiting vegetables/cucurbits, a maximum storage period of 3 days (MITC) and 14 days (DMTU) could be tentatively applied to pepper.
- For the generation of new residue trials, time between sampling and analysis should be kept as short as reasonably achievable and appropriate anti-oxidants should be added prior to sample homogenisation to improve stability of DMTU.

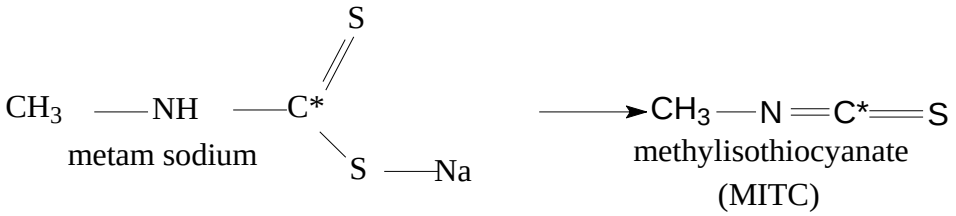
The storage periods of supervised residue trial samples prior to analysis, and the possible impact thereof on the reliability of the trial results, have been discussed in section **2.7.4**.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

2.7.2.1 Plants

Metam-sodium acts as a soil fumigant (nematicide, fungicide, herbicide and insecticide) through rapid degradation to Methyl Isothiocyanate (MITC), which is active on living organisms present in the soil at the time of the application. Therefore, MITC is a known and expected main metabolite formed in soil from the parent compound metam, which thus serves as a precursor.

All available metabolism studies discussed further below were conducted using N-methyl-^{14}C-(thiocarbonyl)-metam-sodium and with application to bare soil (before planting a crop), in line with the intended use. The radiolabel position and formation of MITC from metam-(sodium) is visualized in the figure here below.



* Position of ^{14}C in the metabolism studies.

Note: Besides the sodium variant of the active substance metam, also another variant of metam is supported by one of the applicants (Taminco), namely Metam-potassium (see **1.5.1**). However, based on the fact that it concerns two different inorganic salts of the active substance, which are both precursors of the same biologically active compound (i.e. MITC), it can reasonably be expected that metabolism and uptake in plants grown in treated soil will be similar. Therefore, extrapolation between metam-sodium and metam-potassium is considered acceptable. With regard to application dose, the dose expressed as metam is deemed important, as this determines how much MITC can be formed.

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), the metabolism of metam was investigated – using ^{14}C-(thiocarbonyl)-radiolabelled metam – for soil injection or drip-irrigation in various crops (radishes, turnips, Chinese cabbage and tomatoes), representative of three crop categories (i.e. root (and tuber) vegetables, leafy crops and fruit crops) (BE, 2010b; EFSA, 2011). Whereas the study on turnip was conducted at an application rate (356 kg metam-sodium/ha) covering the representative uses (2.3 N compared to 153 kg/ha on potatoes/carrots/onions supported by Lainco), the 3 older studies on radish, Chinese cabbage and tomatoes were underdosed (at 40 kg metam-sodium/ha).

Note: During the original peer review, following justifications and arguments were provided by the applicant Taminco for the lower dose rates applied in the radish, tomato and Chinese cabbage metabolism studies. It was claimed that the application rate of 40 kg a.s./ha could be considered equivalent 400 kg a.s./ha and therefore simulated usual agricultural application rates.

- It was argued that the intended higher application rates could not be used for the metabolism studies for the following reasons:
  - The liberation of MITC in this" open system" would be too dangerous for the greenhouse workers (cf. protection against radioactive radiation). The volatilisation starts immediately after mixing the Metam-sodium with the soil.
  - Restrictions by the authority: using the radioactive test substance at the normal application rate would exceed the limit of total radioactivity the applicant is allowed to handle in the isotope greenhouse at the agricultural research centre.

- It was claimed that this does not imply differences in the dose in the soil at the time of sowing/transplanting the crop because of the adapted waiting period and the use of a bio-assay in practice before planting a crop on the treated soil. The bioassay (cress test) uses cress seeds that are very sensitive to volatile phytotoxic compounds. The treated soil samples as well as controls with untreated soil are incubated at room temperature for 2-3 days. If no difference in the germination is observed the culture can be planted. Otherwise the aeration time has to be extended until the cress test showed negative results.

- In the study reports of the underdosed metabolism studies, reference was made to trials performed prior to the start of these metabolism studies. It was mentioned that in those pre-trials, a *"similar acting soil fumigant"* was incorporated into soil that had been filled into a container. The container was then immediately sealed air tight. 7 days after the start aliquots of the soil were sampled and the radioactivity was determined by radio combustion analysis followed by Liquid Scintillation Counting. These results apparently demonstrated that the starting concentration at an application rate of 5 g a.s./m^2 (soil depth 20 cm) in the model system was similar to the total radioactive residue levels recovered in open containers determined 7 days after a more critical (10x) dose rate of application of 50 g a.s./m^2 (Dr. [redacted], BASF, Personal communication).
  However, neither further experimental details nor results were reported and provided to the RMS. Hence, it is difficult to validate or invalidate the claim.

Overall, the RMS is of the opinion that the claim and assumption that a reduced application rate could lead to almost the same radioactive residues levels in soil (at planting) as compared to the normal application rate according to the representative uses, cannot be confirmed on the basis of the data provided.

Another shortcoming of the older metabolism studies (turnip, radish, Chinese cabbage and tomatoes) was that samples were apparently stored for a long period before analysis, and the impact thereof on the representativeness of the determined nature (and magnitude) of radioactive residues in the samples analysed is unknown.

A summary of the findings of the older – previously evaluated – metabolism studies is presented here below. Some additional considerations have been made by the RMS following a new review of the study reports (in the framework of the assessment of the application for renewal of a.s. approval). Overall, these studies are to be considered as supportive information only.

The studies on radish, Chinese cabbage and tomatoes all showed that soil treatment with radiolabelled metam-sodium at an application rate of 40 kg a.s./ha (soil incorporation at 20 cm depth) leads to significant total radioactive residues (TRR) in the crop parts at harvest, i.e.

- in roots and leaves of radishes sown in the treated soil 31 DAT (and harvested 63-80 DAT);
- in leaves of Chinese cabbage planted in the treated soil 33 or 104 DAT (and harvested 77 and 104 DAT, resp.);
- in green plant parts and immature and mature fruits of tomato plants planted in the treated soil 15 DAT (and harvested 92 DAT).

The significant uptake of residues from the soil by the plants (and significant translocation to the upper plant parts) was also observed in the metabolism study with turnips (after soil drench treatment at 356 kg metam-sodium/ha).

In the studies on radish, Chinese cabbage and tomatoes, the radioactive residues were mainly characterised as small, polar and aqueous soluble compounds. None of the radioactive residues could be identified. Metam, MITC, DMTU and other methyl(thio)urea compounds could not be detected.
However, it is to be noted that identification attempts were limited to TLC (Thin Layer Chromatography) analysis at the time of the study, which is rather unspecific. Furthermore, resolution of radioactive peaks in TLC analysis was rather poor. Therefore, it is difficult to derive any further robust conclusions from these studies.

Some further attempts to characterize and identify residues were made in the turnip metabolism study, *i.a.* by means of derivatization steps to confirm incorporation into sugars and use of subsequent hydrolysis with enzymes selective for cellulose, starch, protein and pectin and finally lignin extraction. The readily extractable radioactive residues comprised mainly polar components, which were tentatively characterized as peptides and carbohydrates (mono-, oligo- and polysaccharides). Incorporation of radioactivity into reducing sugars was confirmed. Non-extractable residues were distributed over a variety of natural products such as cellulose, hemicellulose, starch, proteins, pectin and lignin. These experiments indicated that the majority of the radioactivity is incorporated into the carbon pool of the turnip crop (probably and at least via glucose metabolism).
However, whereas it had been concluded by the study authors in the study report ([redacted], 1995) and subsequently concluded in the framework of the previous EU peer review of metam (under Dir. 91/414/EEC) that "*none of the different metabolites fractions separated by TLC analysis co-chromatographed with either the parent or the parent related metabolites including MITC"* (see DAR – BE, 2010b) and *"No degradation of the parent compound into its substituted thioureas or methylated ureas was observed."* (EFSA, 2011), a new review of the study report in the framework of the renewal application revealed that this was not fully correct, since at least and particularly metabolites 1 and 2 had retention times that were very similar to

those of parent-related compounds (metam/MITC and DMTU, respectively). Metabolite 1 accounted for 9.7 %TRR (0.27 ppm eq.) and 3.5 %TRR (0.083 ppm eq.) in turnip tops and roots, respectively. Metabolite 2 accounted for 3.3 %TRR (0.094 ppm eq.) in turnip tops. As a consequence, although it is acknowledged that the study showed a large incorporation of radioactivity into natural components of the crop, the findings of the study do not allow concluding that the observed unidentified fractions – which may represent metabolites at significant absolute level – are out of concern. More specifically, although no unequivocal identification could be performed in the study (due to low specificity of TLC analysis set-up), the presence of parent-related metabolites, such as MITC (and/or metam) and/or 1,3-DMTU, at significant levels (>0.01 mg/kg) in turnip root and leaves cannot be excluded on the basis of the results of this metabolism study.

In the framework of the application for renewal of approval, the second (new) applicant Lainco provided results of additional, GLP-compliant plant metabolism studies, in which ^{14}C-(thiocarbonyl)-radiolabelled metam-sodium was applied by soil injection.

In the study on **potatoes** soil treatment with radiolabelled metam-sodium at an application rate of 156 kg a.s./ha (soil incorporation at 25 cm depth and sealing of soil surface by compaction) resulted in radioactive residues (1.3-2.4 mg/kg metam-sodium eq.) in tubers from potatoes sown in the treated soil 29 DAT (tubers harvested 114 DAT).
Characterisation of the residues indicated incorporation of radioactivity into natural products, i.e. readily extractable carbohydrates, plant cell wall carbohydrates and, to a lesser extent, starch. No incorporation into lignin was detected and no release of volatile radioactivity was detected. Metam sodium, N,N-dimethylthiourea (DMTU) and N,N-dimethyl urea (DMU) were not detected in the study. As no reference standard for MITC was used in this study, no robust conclusion as regards its absence can be derived from the study results.

In the study on **lettuce** soil treatment with radiolabelled metam-sodium at an application rate of 380 kg a.s./ha (soil incorporation at 5 cm depth and covering with black plastic PE sheet) resulted in radioactive residues (4.5-6.3 mg/kg metam-sodium eq.) in leaves of lettuce plants that were transplanted in the treated soil 21 DAT (leaves harvested 69 and 92 DAT).
Characterisation of the residues indicated incorporation of radioactivity into natural products, including proteins, lignin, hemicellulose and cellulose. Chromatographic analysis of extractable residues showed polar metabolites, which were however not identified. Metam-sodium and DMTU were concluded to be absent (based on co-chromatography). As no reference standard for DMU was used in this study, no robust conclusion as regards its absence can be derived from the study results.

In the study on **tomatoes** soil treatment with radiolabelled metam-sodium at an application rate of 421 kg a.s./ha (soil incorporation at 15-20 cm depth) resulted in residues (9-13 mg/kg metam-sodium eq.) in mature fruits of tomato plants planted in the treated soil 20 DAT (fruits harvested 41 and 61 DAT). Residues were in part characterised as polar compound(s), but none of the radioactive residues could be identified. Metam, MITC or methylamine were not detected by TLC, noting however that detection of ^{14}C-methyl amine would anyhow not be expected on the basis of the ^{14}C-radiolabel position. No reference standards were used for DMTU and other methyl(thio)urea in this study, so no robust conclusion as regards absence of DMTU and DMU can be derived from the study results. Two major components were observed in tomato fruit extracts, accounting for 38%TRR and 14%TRR, respectively, but their identity remains unknown; no further attempts to characterise or identify those components were made in the study (although required by OECD TG 501). The study is therefore considered as supportive information only.

Overall, **RMS concludes** that no clear degradation pathway of metam-sodium could be depicted and the nature of all residues in crops planted/sown in treated soil remains somewhat uncertain, since the identity of the radioactive residues in the crops could not be elucidated in any of the metabolism studies. However, in all studies in which attempts were made to characterize the radioactive residues, the findings indicated an extensive metabolism of metam-sodium to small, polar metabolites entering the plant's carbon pool and incorporation into a wide range of natural plant products. Neither parent compound, nor its metabolites MITC and DMTU were identified as such in any of the crops, although it should be noted that their presence could not be fully ruled out (due to low specificity of TLC analysis in the older metabolism studies and/or the choice of reference standards in the more recent metabolism studies). In addition, samples from lettuce were apparently stored for a long period (up to 8 months) before analysis, and sample duration in the potato study was not reported, so the impact thereof on the representativeness of the determined nature (and magnitude) of radioactive residues in the samples analysed is unknown.

Nevertheless, it had been previously concluded that as long as no other significant metabolites (which might be taken up by plants) in soil have been identified by the fate and behaviour assessment, then the plant metabolism studies could be accepted (cf. EFSA, 2011). The new plant metabolism studies do not alter the previous assumptions. In the framework of the assessment with a view on renewal of approval, the updated evaluation performed in the area of fate and behaviour has also confirmed that no other significant metabolites (other than MITC and/or DMTU) are present – see **2.8.1** – and therefore the conclusion that further plant metabolism data are not required, remains valid.

2.7.2.2 Animals

Studies investigating the nature of residues in animals exposed (via their diet) to possible residues of metam (including its metabolites MITC and DMTU) are not available.

Taking into account the authorised uses of metam, it appeared that livestock metabolism data may be required for DMTU and possibly also for MITC (if missing data are provided for all crops); see data gap identified in the framework of MRL review (EFSA, 2019a).

In the framework of the application for renewal of approval, no new data were provided; the need for animal metabolism studies was waived by both applicants, based on livestock dietary intake considerations taking into account only the representative uses; see here below and also in section **2.7.5.1**.

TAMINCO:
The need for livestock or fish metabolism studies has been acceptably waived by the applicant Taminco, with reference to the fact that the representative crops (lettuce, baby leaves and ornamentals) are not used as livestock or fish feed items (according to the EFSA animal model 2017 and SANCO/11187/2013 rev.3, respectively); see also section **2.7.5.1**.

LAINCO:
A more specific waiver was provided by Lainco (cf. potato and carrots as possible feed items); see section **2.7.5.1**. RMS concludes that, on the basis of the information currently available, a livestock dietary burden above the legal trigger of 0.004 mg/kg b.w./day cannot be excluded, neither for MITC, nor for DMTU and therefore, waiving of the need for livestock metabolism studies is not accepted on this basis **(data gap;** relevant for the representative uses on potatoes and carrots).

2.7.3 Definition of the residue

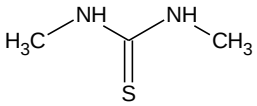
A comparative summary of the residue definitions for metam is presented in **Table 2.7.3-1**, showing the current proposals (in the framework of renewal of a.s. approval), the previous proposals (in the framework of the original EU peer review) and the current legal residue definitions (for monitoring) under the MRL regulation.

Table 2.7.3-1: Overview of residue definition(s) (proposals) for metam in different EU evaluation frameworks

	DAR (BE, 2010b) / EFSA, 2011 ***Dir. 91/414/EEC*** (*)	**EFSA, 2019a** ***Reg. (EC) 396/2005*** (**) → *Reg. (EC) No 149/2008*	**DRAR (BE, 2021)** ***Reg. (EC) 1107/2009*** (***)
Plant			
Monitoring	MITC	MITC (resulting from the use of dazomet or metam)	MITC (resulting from the use of dazomet or metam)
Risk assessment	MITC	1) MITC 2) DMTU	1) MITC 2) DMTU
Animal (tissues, poultry eggs, milk)			
Monitoring	*Not required*	Inconclusive	Inconclusive
Risk assessment		Inconclusive	Inconclusive

MITC: methyl isothiocyanate

DMTU: N,N'-dimethylthiourea



2.7.3.1 Commodities of plant origin

(*) Previous EU peer review of metam

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), **MITC** was considered to be the most appropriate residue definition for risk assessment and monitoring. The inclusion of this compound, although it had not been detected in plant metabolism studies, was justified on the basis of the fact that MITC is actually the biologically active compound generated from the precursor metam and is therefore expected to be the predominant metabolite in soil (for possible uptake by plants). Furthermore, MITC was found to be more (acutely) toxic than the parent compound metam (EFSA, 2011). Inclusion of the parent itself was not deemed necessary, as the available metabolism studies showed an extensive metabolism into small, polar metabolites entering the plant's carbon pool and incorporation into a wide range of natural plant products, and because metam itself could not be identified either in any of the metabolism studies (*vide supra* – **2.7.2.1**).

Despite the shortcomings of the available metabolism studies, it was concluded that as long as no other significant metabolites (which might be taken up by plants) in soil have been identified by the fate and behaviour assessment, then the plant metabolism studies could be accepted (cf. EFSA, 2011).

Because the application rates of the active substance metam are so high (compared to usual rates for most other active substances), further consideration was also given to impurities in the technical grade active ingredient, which are also released in the environment at high (kg/ha) rates and, if taken up by crops, may result in residues in edible commodities at crop harvest; see DRAR **Vol.4, C.1.4** (Toxicological assessment of metam impurities).

In particular the relevant organic impurity **DMTU** (N,N'-dimethylthiourea) was considered further for possible consumer exposure/risk.

- DMTU was not observed as a rat metabolite and limited toxicological information was available (see **2.6.1**). Hence, its toxicity was considered equivalent to the toxicity of the parent compound metam, based on chemical structure, based on the lack of data on DMTU and considering the toxicological properties of the parent compound. Since metam-sodium was tested with batches containing 1% of DMTU, it was considered that all toxicity endpoints and related classifications sufficiently covered the toxicity of this impurity. (cf. EFSA, 2011)
- There were indications that DMTU is also a metabolite/degradation product formed in the environment after application of metam-sodium (or metam-potassium) aqueous solution (cf. observation of DMTU in MITC hydrolysis study (see DRAR Vol.3, B.8.2.1.1) and in metam water/sediment study (see DRAR Vol.3, B.8.2.2.3). DMTU may be formed by reaction of MITC with methylamine (CH_3NH_2).
- However, as additional supervised residue trials performed on pepper, tomato, carrot, eggplants and cucumber showed a <LOQ residue situation (<0.01 mg/kg) for DMTU, the compound was eventually not included in the residue definition at that time.

(**) MRL review

The review of existing EU MRLs for metam in accordance with art.12 of Reg. (EC) No 396/2005 was finalised in 2019 (EFSA, 2019a). In that framework, results from additional supervised residue trials (from the other applicant Lainco – see **2.7.4** further below) were considered and those revealed the possible occurrence of both MITC and DMTU at quantifiable levels in different plant commodities. Hence, the previously assumed <LOQ residue situation for both compounds was challenged, and a reconsideration of the residue definitions was needed.

As a consequence, the inclusion of **MITC** in the residue definition (for both risk assessment and monitoring) was confirmed.
In addition, it was concluded to consider **DMTU** in a separate residue definition, at least for risk assessment. The source of the significant occurrence of DMTU observed in residue trials performed with metam was not fully elucidated. It can be due the original content of the relevant impurity in the technical material metam, but it is not excluded that DMTU may also be released from the breakdown of metam in soil. One hypothesis is that DMTU might be a soil degradation product formed by the reaction of MITC with methylamine (see also above). (cf. EFSA, 2019a).

Note: The current residue definition for MRL enforcement (as legally established in Reg. (EC) No 396/2005) is 'MITC (resulting from the use of dazomet or metam)'. RMS recalls that MITC is a common metabolite that is also generated by the active substance dazomet (another MITC-generating soil disinfectant).

(***) EU peer review for renewal of approval

In the framework of the application for renewal of approval, being the scope of the current assessment, the available additional plant metabolism studies were considered (see **2.7.2.1**), but those metabolism studies do not alter the previous assumptions with regard to overall metabolism. Additional residue trials analysing for MITC and DMTU were also provided and MITC and/or DMTU were found at quantifiable levels in several trials and in several crops, although at lower levels compared to previous trials (see **2.7.4** further below). However, the results confirmed that it is appropriate to consider both components for risk assessment.

The updated evaluation performed in the area of fate and behaviour has also confirmed that no other significant metabolites (other than MITC and/or DMTU) are present – see **2.8.1**.

As a consequence, RMS proposes to maintain the same residue definitions as derived during MRL review.

With regard to monitoring, the RMS further notes that there are indications from the public literature on the possible occurrence of MITC in some foods of plant origin:

- MITC may also be used (illegally) as an antifermentative agent in wine, which is reflected by several publications in the open literature on analytical methodologies to determine MITC residues in wine.[6]

- MITC may also occur naturally in some particular plant matrices used as human food. As a matter of fact, it may be formed by enzymatic conversion of its precursor glucocapparin, which is one of many glucosinolates.
  - Glucosinolates in general are naturally occurring secondary metabolites found mainly in some species belonging to the order *Brassicales*. These S-linked glucosides (S-glucopyranosyl thiohydroximates) are converted into an isothiocyanate (besides other reaction products) upon hydrolysis of the thioglucoside bond by an endogenous enzyme called myrosinase. A figure showing the formation of *i.a.* isothiocyanates in general by the glucosinolate-myrosinase system is shown here below.[7] It has been presumed that the myrosinase enzyme and the glucosinolates are stored in different cells, either in the same or in different subcellular compartment in intact plants.[8] Hence, the two components only come in contact with each other in case of cellular disruption when the plant tissue is damaged (e.g. by insect attack, chopping or chewing).

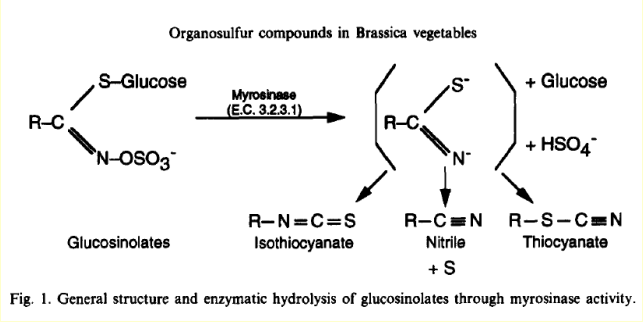


Fig. 1. General structure and enzymatic hydrolysis of glucosinolates through myrosinase activity.

  - Glucocapparin was reported to occur in plants belonging to the caper family (*Capparaceae*), such as:
    - *Boscia senegalensis*, a traditional food plant in Africa, whose seeds (also known as hanza, aizen) are consumed, however after having been cooked or soaked with water to remove bitter (and potentially toxic) components. MITC was identified as the main degradation product of glucocapparin extracted from leaves and fruits of *B. senegalensis*[9] and MITC was also found in the waste water from the soaking process to prepare hanza.[10]
    - *Capparis spinosa*, of which the immature flower buds are part of the human diet (capers – code 0850020 in Reg. (EC) No 396/2005).[11,12]

[6] Gandini and Riguzzi, 1997 (*J. Agric. Food Chem.* 1997, 45, 8, 3092–3094); Uchiyama *et al.*, 1992. (*Food Hygiene and Safety*. 1992 Volume 33 Issue 6 Pages 603-608_1); Satto *et al.*, 2020 (*Journal of AOAC International*, Volume 77, Issue 5, 1 September 1994, Pages 1296–1299)

[7] Stoewsand, 1995. Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables – A review *Fd Chem. Toxic. Vol. 33, No. 6, pp. 537-543*

[8] Kissen *et al.*, 2009. The 'mustard oil bomb': not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochem Rev (2009) 8:69–86*

[9] Gueye *et al.*, 2013. Development of a performant method for glucocapparin determination in Boscia senegalensis Lam Ex. Poir.: A study of the variability. *American Journal of Analytical Chemistry, 2013, 4, 104-110.* https://www.scirp.org/pdf/AJAC_2013022709223245.pdf

[10] Rivera-Vega *et al.*, 2015. Allelopathic effects of glucosinolate breakdown products in Hanza [*Boscia senegalensis (Pers.) Lam.*] processing waste water. *Front. Plant Sci., 14 July 2015.* https://doi.org/10.3389/fpls.2015.00532

[11] Bianco *et al.*, 2012. Identification of glucosinolates in capers by LC-ESI-hybrid linear ion trap with Fourier transform ion cyclotron resonance mass spectrometry (LC-ESI-LTQ-FTICR MS) and infrared multiphoton dissociation. *J. Mass Spectrom. 2012, 47, 1160–1169*

[12] Matthäus *et al.*, 2005. Glucosinolates and Fatty Acid, Sterol, and Tocopherol Composition of Seed Oils from Capparis spinosa Var. spinosa and Capparis ovata Desf. Var. canescens (Coss.) Heywood. *J. Agric. Food Chem. 2005, 53, 7136−7141*

Note: Also claims were found in open literature that glucocapparin be present in some species of the *Brassicaceae* family, such as horseradishes and cauliflower[13]. However, the basis of these claims could not be verified by the RMS.

2.7.3.2 Commodities of animal origin

In the framework of the previous EU peer review of metam (EFSA, 2011), it was not deemed necessary to establish a residue definition for products of animal origin, because of the presumed <LOQ residue situation in commodities of plant origin.

However, in the framework of the MRL review (EFSA, 2019a), it was concluded that further investigation on the nature (and magnitude) of residues in livestock commodities is required, in particular regarding the indicative estimation of livestock dietary burden for DMTU, which already exceeded the trigger value.

In the framework of the application for renewal of approval, still no new data were available (see **2.7.2.2**). Therefore, the data gap identified in the framework of MRL review remains unfulfilled and the residue definitions for animal products remain 'inconclusive'.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Initially, in the framework of the initial EU peer review of metam (under Dir. 91/414/EEC), merely results of field residue trials conducted in the USA were provided by the main data submitter (Taminco) for a wide range of crops, i.e. snap beans, cucumbers, potatoes, mustard greens, peppermint, basil, tobacco, Cantaloupes, radish, green onion, leaf lettuce, head lettuce, spinach, turnip, broccoli, sweet corn, bulb onion, garlic, strawberry, tomato and cabbage (see references IIA 6.3/01 – IIA 6.3/21 in Vol.2 of the DRAR). However, it was concluded that these trials could not be relied upon, *i.a.* due to shortcomings of the analytical method (validation), sample storage duration and due to the fact that the trials were all conducted outside Europe (BE, 2007/2008; EFSA, 2008). Therefore, these trials have not been reconsidered and are no longer reported in the DRAR.

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), representative soil application uses on several crops were assessed, i.e. carrot, lamb's lettuce, cucumber, aubergine, pepper, potato, strawberry, tomato and grapes, but only a limited number of EU residues field trials was available to support the claimed and expected 'no residue situation' for both metabolites, MITC and DMTU (BE, 2010b; EFSA, 2011).

In the framework of the review of existing EU MRLs for metam in accordance with art.12 of Reg. (EC) No 396/2005, results from additional supervised residue trials (from a second applicant, Lainco) revealed the possible occurrence of both MITC and DMTU at quantifiable levels in different plant commodities (see also below). As a consequence, the previous hypothesis that residues of MITC and DMTU would always be <LOQ in harvested commodities was rejected and a reconsideration of the minimum required number of residue trials was needed (EFSA, 2019a).

In the framework of the application for renewal of approval, being the scope of the current assessment, two applicants supported representative soil treatment uses with more precisely described use conditions (which includes the mandatory use of a Total Impermeable Foil (TIF)) and in most cases consisting of lower application dose rates. New residue trials were provided in support of the new intended uses. A brief summary for each of the applicants is given below.

- **TAMINCO:** Soil treatment (under protection) with metam-sodium or metam-potassium before planting/sowing lettuces or baby leaf crops is supported; see GAP details further below. The new residue trials provided were all performed with metam-sodium. However, extrapolation between metam-sodium and metam-potassium is considered acceptable (see **2.7.2.1**) and furthermore, the intended application rates for metam-sodium and metam-potassium correspond to the same application dose when expressed as metam (acid). Therefore, no further particular consideration of the potassium variant is deemed necessary for the representative uses on lettuce and baby leaf crops. The provided data are sufficient to derive risk assessment values (STMR, HR) for both MITC and DMTU and for MRL setting.

- **LAINCO:** Soil treatment of metam-sodium before planting potato, carrot, onion (outdoor, in countries belonging to the Southern European residue zone only, so excluding FR) and pepper (under protection) is supported; see GAP details further below. Lainco had already performed its own residue trials after the initial approval of metam. These trials were submitted at national level within the product data package in the

[13] Puri, B. and Hall, A., 1998. Phytochemical dictionary: a handbook of bioactive compounds from plants, second edition, 16 Dec. 1998.

framework of reregistration following approval of metam under Dir. 91/414/EEC (step 2). Spain was the evaluating RMS for the Step 1 and 2 for Lainco S.A's data package. Quantifiable residues for MITC and/or DMTU were observed in the Lainco data package[14], while the Taminco dataset had showed a <LOQ situation for similar uses.

With respect to the representative uses supported by Lainco with a view on renewal of a.s. approval, Lainco provided new trials, but these alone are deemed insufficient following review by the RMS and further fully GAP-compliant and valid residue trials are required (**data gap**).
Moreover, Lainco waived the relevance of the positive findings in the older trials as follows [certain parts underlined by RMS]:

<<
It is believed that the lack of superficial ploughing of the treated field prior to the sowing / planting of the crops led to the detection of the residues within the harvested crop samples. The GAP support[ed] at renewal indicates that *"After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked in order to allow aeration of any remaining MITC residues and to prepare the soil for sowing or planting"*. Therefore, new residue trials on potato, carrot and onion were performed, which provide analyses of the MITC and DMTU residues within the crop fraction with a reduced interval between sampling and residue extraction of 2 – 3 days. They also provide a realistic residues profile in crops following the treatment of the field according to the intended GAP and include side by side field trials with and without soil reworking/aeration to test the impact of soil working on residue levels**.** These new trials were not available and not evaluated during the EU MRL review (EFSA, 2019).
>>

However, RMS is of the opinion that this justification is **not** acceptable; the case made by the applicant is insufficiently supported by data. RMS concludes that no convincing evidence is available that could justify the omission of the quantifiable residue findings in the older residue trials.

- The claimed *"lack of superficial ploughing of the treated field prior to the sowing/planting of the crops"* in the trials with positive residue findings – a correlation between these two aspects is suggested by the applicant – does not appear supported by any detail reported in the study reports; on the basis of the study report, it cannot be excluded that some reworking of the soil was done prior to sowing. Moreover, for trial S165 – notably the trial in which the highest residue levels for MITC were measured in potatoes at harvest – it was reported that 'rotary ploughing' was carried out 3 days before drilling of the potato tubers. In addition, positive residue findings were also found in carrot trials where tillage was performed a few days before seeding.
- The too long sample storage periods prior to analysis are mentioned by the applicant as one of the main reasons for considering the older trials (with positive residue findings) as unreliable. However, longer storage stability periods implicate an underestimation of the actual residue levels in case degradation upon storage would have occurred. Therefore, this argument is considered highly questionable and considered very weak for ignoring the positive residue findings.
- The new set of trials on potato and carrots included a few side-by-side experiments with use of TIF (Total Impermeable Foil) on one plot and without use of TIF on another plot, but due to the fact that residues were either not found at quantifiable levels or at very low level in these side-by-side trials and also taking into account differences in application rates, no conclusion can be drawn from these data; it is not possible to attribute the occurrence of MITC residues in potato tubers in one trial to the use of the TIF (or soil reworking). The influence of the absence of a soil coverage with TIF between application and crop transplanting on the amount of residues of MITC and DMTU in the soil is unknown. It cannot be excluded that use of TIF would lead to more residues available in soil at transplanting and even more uptake by the crop transplanted.

The RMS concludes that reliable data or information that could reasonably justify the omission of the available supervised residue trials on potatoes, carrots and onions in which (relatively high) quantifiable levels of MITC and DMTU were observed, are missing (**DATA GAP**).

[14] RMS notes that in 2014, Lainco submitted an MRL application to increase several EU MRLs (cf. EFSA-Q-2016-00117), but the proposed MRL amendments were rejected based on issues concerning storage stability and eventually, Lainco withdrew its MRL application (on 21/10/2020).

The available data are discussed further for each representative crop in the following subsections (**2.7.4.1 – 2.7.4.7**). As regards the judgement on the validity and required number of residue trials, the assessment was based on the requirements stipulated in Reg. (EU) No 283/2013 and in EC guideline SANCO 752/VI/95-rev. 10.3 (European Commission, 2017)[15].

[15] European Commission, 2017. Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95-rev. 10.3. 13 June 2017

2.7.4.1 Lettuces (TAMINCO)

The representative GAPs relevant for residues are summarised below.

Crop	F G or I	Application			Application rate Metam Na 510 SL (a.s.=metam-sodium)			PHI (days)	Remarks
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha		
Lettuce	G	drip irrigation	6 weeks before planting	1 application every 3 years	300 (250)*	153 (172)*	15000-60000	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5 - 2.0 % v/v With TIF (6 weeks) Permanent structures

* Application rate expressed for the equivalent potassium salt product 'Metam K 690 SL'

Nine (9) new and valid supervised residue trials are available in support of this GAP. These trials are considered GAP-compliant and valid, as they were conducted under greenhouse conditions in Europe with soil treatment (via drip irrigation) at an application rate of 152-164 kg metam-sodium/ha, followed by soil coverage (with TIF) for about 6 weeks (40-50 days), subsequent superficial tillage, a cress test and lettuce planting. Analyses were performed rapidly after sampling – extraction generally within 48 hours after sampling (only 2 exceptions for DMTU; 49 days and 70 days) – using fully validated analytical methods. Therefore, the analysis results are deemed sufficiently reliable.

No quantifiable residues of MITC or DMTU were found in the lettuce heads at commercial harvest (LOQ of 0.01 mg/kg for each analyte). Results are summarised below.

Crop	Dossier reference	Study report no	MITC (mg/kg)	DMTU (mg/kg)
Lettuce (indoor)	Taminco – KCA 6.3.1/01	RDE-19-38187	<0.01 (9x)	<0.01 (9x)

Notes:

- 1 trial was disregarded, due to quantifiable MITC residues found in both control and treated lettuce sample. This apparent contamination was also observed in other trials conducted on other crops in the same city in Spain ([redacted]).
- 4 additional indoor trials on lettuce were considered in the framework of the MRL review ([redacted], 2013 – Report No 12055RLE), with a higher application rate of 311 – 341 kg metam-sodium/ha via drip irrigation, 21±1 days before planting, but without TIF covering. Results reported were: MITC: <0.01 (4x); DMTU: <0.01 (3x); 0.019 (cf. Evaluation Report France, 12 June 2017 – FR, 2017). **Thus, even at higher application rates and without TIF covering, quantifiable levels of DMTU in lettuce heads at harvest cannot be fully excluded.** However, this was apparently not correctly reflected in the EFSA Reasoned Opinion EFSA Journal 2019;17(1):5561 (EFSA, 2019), which also reported "4x <0.01" for DMTU.

- A trial on lettuce that had been previously peer reviewed (BE, 2010b; EFSA, 2011) was not any longer considered adequately representative for the representative use supported in the framework of renewal of a.s. approval and has not been considered. No MITC residues were found in this trial. For further details, see Vol.3, **B.7.3.1** (Taminco – KCA 6.3.1/02).

2.7.4.2 Baby leaf crops (TAMINCO)

The representative GAPs relevant for residues are summarised below.

Crop	F G or I	Application			Application rate Metam Na 510 SL (a.s.=metam-sodium)			PHI (days)	Remarks
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha		
Baby leaf	G	drip irrigation	6 weeks before sowing	1 application every 3 years	150-200 (125-167)*	77 – 102 (86-115)*	7500/30000 - 10000/40000	n.a.	Diluted with water directly in the drip irrigation lines at concentration 0.5 - 2.0 % v/v With TIF (6 weeks) Permanent structures

* Application rate expressed for the equivalent potassium salt product 'Metam K 690 SL'

Eight (8) new and valid supervised residue trials are available in support of this GAP. These trials are considered GAP-compliant and valid, as they were conducted under greenhouse conditions in Europe with soil treatment (via drip irrigation) at an application rate of 102-110 kg metam-sodium/ha, followed by soil coverage (with TIF) for about 6 weeks (40-50 days), subsequent superficial tillage, a cress test and sowing of baby leaf crops (lettuce, lamb's lettuce or spinach). Analyses were performed rapidly after sampling – extraction within 32 hours after sampling – using fully validated analytical methods. Therefore, the analysis results are deemed sufficiently reliable.

No quantifiable residues of MITC or DMTU were found in the baby leaves at commercial harvest (LOQ of 0.01 mg/kg for each analyte). Results are summarised below.

Crop	Dossier reference	Study report no	MITC (mg/kg)	DMTU (mg/kg)
Baby leaf crops (indoor)	Taminco – KCA 6.3.2/01	RDE-19-38189	<0.01 (8x)	<0.01 (8x)

Note:

- 1 trial was disregarded, due to quantifiable MITC residues found in both control and treated lettuce sample. This apparent contamination was also observed in other trials conducted on other crops in the same city in Spain ([redacted].

2.7.4.3 Potatoes (LAINCO)

Metam formulated as Metam Sodium 51% SL is intended to be used on potato according to representative GAPs summarised in **Table 2.7.4.3-1**.

Table 2.7.4.3-1: Representative GAPs for metam on potato

Outdoor/ Greenhouse	Number of applications	Application rate	Method of application	Period of application	Remarks
Outdoor (SEU)[16]	1 application every 3 years	153 kg metam-sodium/ha	Soil injection (15-20 cm depth) in combination with the use of Total Impermeable Foil (TIF)	Spring to winter Pre-plant or pre-sowing	Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked. A cress germination test should always be performed prior to sowing or planting.

Five (5) new and valid supervised residue trials are available in support of this GAP in Southern Europe. These trials are considered GAP-compliant and valid, as they were conducted with soil treatment (via soil injection) at an application rate of 148-190 kg metam-sodium/ha, followed by soil coverage for 19-21 days (using TIF), subsequent superficial tillage, a cress test and potato planting 25-28 days after application. Analyses were performed rapidly after sampling; in most cases within 3 days and in any case not beyond the maximum period of demonstrated stability for MITC (7 days) and DMTU (197 days with addition of anti-oxidant) in potatoes. Anti-oxidant was added to the samples to improve stability of DMTU. Therefore, the analysis results are deemed sufficiently reliable.

No quantifiable residues of DMTU were found in the potato tubers at commercial harvest, whereas MITC was measured in one trial, up to 0.015 mg/kg. LOQ was 0.01 mg/kg for each analyte. Results are summarised below.

Crop (region)	Dossier reference	Study report no	MITC (mg/kg)	DMTU (mg/kg)
Potatoes (SEU)	Lainco – KCA 6.3.1/03	RDE-19-38138	<0.01 (4x), 0.015	<0.01 (5x)

The applicant announced that 3 additional residue trials on potatoes in Spain were planned in 2020, to complete the SEU trial data set for potatoes. The RMS proposes to request and assess these additional trial data during the peer review (**DATA GAP**).

Notes:
- 1 new trial was disregarded, due to quantifiable MITC residues found in both control and treated potato sample. This apparent contamination was also observed in other trials conducted on other crops in the same city in Spain ([redacted]).

[16] In the applicant's dossier (Doc N2 Metam _v4.0 Oct2020), it was initially mentioned that uses on onions (and also on potatoes and carrots) are intended in the following EU Member states: BG, CY, EL, ES, FR, HR, IT, PT. As FR was included in that list, residue trials in the NEU residue zone would normally also be required to support this use in FR. However, as only new trials were provided for onion, the applicant Lainco clarified, at request of the RMS, that the addition of FR was a mistake in final dossier preparation, as it appeared there were insufficient data available to support the use in FR. As a consequence, only the intended use in EU countries belonging to the Southern European residue zone has eventually been considered by the RMS.

- In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), no valid residue trials on potatoes were provided (cf. EFSA, 2008; EFSA, 2011). However, it is noted that additional trials (2 NEU, 1 SEU) on potatoes were submitted by the company Taminco, in the framework of the MRL review according to art. 12 of Reg. (EC) No 396/2005 ([redacted], 2010-2011 – Report No 10054RPO; cf. Evaluation Report – BE, 2013). However, in those trials, application was by soil injection "before winter" (October or December)[17] and sowing of potatoes was much later (83-197 days after application). Therefore, these trials cannot be considered representative of an intended GAP with potato seed drilling just a few weeks after application (as intended by the applicant Lainco – see above). Hence, the results of those trials (<0.01 mg/kg for MITC and DMTU) cannot be relied upon.

- It should be noted that additional sets of trials (4 NEU and 8 SEU) are available on potato. Those trials were performed in 2012-2013, with an application rate around the intended 153 kg metam-sodium/ha. They were submitted at national level after first a.s. approval and were later considered during MRL review (EFSA, 2019a), where it was concluded that those trials are not valid, due to concerns on the storage period of the samples. Indeed, sample storage periods were much too long (and storage conditions inappropriate), particularly considering the rapid decline of MITC and DMTU residue levels observed in storage stability investigations. As a consequence, actual residue levels in potato tubers at harvest had very likely been strongly underestimated by the measurements undertaken. Still, quantifiable residue levels of MITC (0.013 – 0.055 mg/kg) had been found in all the trials conducted in SEU (with application in January – February) and also DMTU had been found above LOQ in several of those SEU trials (up to 0.084 mg/kg).

  According to the applicant Lainco, those positive residue findings can be ignored due to the sample storage issue and due to some other deviations from the representative GAP, such as the absence of soil covering with TIF, no or limited soil re-working and the absence of a cress test before seed drilling. However, the RMS does not agree with the conclusions of the applicant that the positive residue findings should be ignored for these reasons; the deviating design of these trials and the identified shortcomings do not necessarily represent a more critical situation from a residue perspective; more details: see **Vol.3, B.7.3.3** – Lainco – KCA 6.3.1/03.
  It is noted that MITC or DMTU were not found in the older trials conducted in NEU (with application in May, i.e. just like most of the newer trials of KCA 6.3.1/03). It is unclear on the basis of the available data, but it cannot be excluded that the timing of application has played a role and could partially explain the differences between the results of the NEU trials and the SEU trials.

  For the sake of completeness, the results from the SEU trials with positive findings – serving as relevant supplementary information – are summarised here below:

Crop (region)	***Dossier reference***	***Study report no***	***MITC (mg/kg)***	***DMTU (mg/kg)***
Potatoes (SEU)	*Lainco – KCA 6.3.1/01*	*11104RPO*	*0.013, 0.015, 0.031, 0.031, 0.035, 0.036, 0.043, 0.055*	*<0.01 (4x), 0.011, 0.015, 0.073, 0.084*

[17] Trials I106 (IT), GB107 (UK), B108 and B109 (BE). Application was made in period October – December, also for BE trials (date of treatment was erroneously reported as June in Evaluation Report (BE, 2013).

2.7.4.4 Carrots (LAINCO)

Metam formulated as Metam Sodium 51% SL is intended to be used on carrot according to representative GAPs summarised in **Table 2.7.4.4-1**.

Table 2.7.4.4-1: Representative GAPs for metam on carrot

Outdoor/ Greenhouse	Number of applications	Application rate	Method of application	Period of application	Remarks
Outdoor (SEU)[18]	1 application every 3 years	153 kg metam-sodium/ha	Soil injection (15-20 cm depth) in combination with the use of Total Impermeable Foil (TIF)	Spring to winter Pre-plant or pre-sowing	Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked. A cress germination test should always be performed prior to sowing or planting.

Seven (7) new and valid supervised residue trials are available in support of this GAP in Southern Europe. These trials are considered GAP-compliant and valid, as they were conducted with soil treatment (via soil injection) at an application rate of 148-191 kg metam-sodium/ha, followed by soil coverage for 19-21 days (using TIF), subsequent superficial tillage, a cress test and carrot seed sowing 24-28 days after application. Analyses were performed rapidly (max. 4 days for DMTU; max. 3 days for MITC) after sampling with addition of the anti-oxidant to improve stability of DMTU. Therefore, the analysis results are deemed sufficiently reliable.

No quantifiable residues of MITC were found in the carrot roots at commercial harvest, whereas DMTU was found in two trials, up to 0.06 mg/kg. LOQ was 0.01 mg/kg for each analyte. Results are summarised below.

Crop (region)	Dossier reference	Study report no	MITC (mg/kg)	DMTU (mg/kg)
Carrots (SEU)	Lainco – KCA 6.3.2/04	EGL-19-38139	<0.01 (7x)	<0.01 (5x), 0.02, 0.06

On the basis of these data, the applicant Lainco argues that a "no residue situation" can be concluded on for MITC in carrot and that no further data be required to support the representative use on carrot. However, RMS notes that 1 valid GAP-compliant trial is still missing to complete the minimum dataset for DMTU (**data gap**). The applicant announced that 2 additional residue trials on carrots in Spain were planned in 2020, to complete the SEU trial data set for carrots. The RMS proposes to request and assess these additional trial data during the peer review (**DATA GAP**).

[18] In the applicant's dossier (Doc N2 Metam _v4.0 Oct2020), it was initially mentioned that uses on onions (and also on potatoes and carrots) are intended in the following EU Member states: BG, CY, EL, ES, FR, HR, IT, PT. As FR was included in that list, residue trials in the NEU residue zone would normally also be required to support this use in FR. However, as only new trials were provided for onion, the applicant Lainco clarified, at request of the RMS, that the addition of FR was a mistake in final dossier preparation, as it appeared there were insufficient data available to support the use in FR. As a consequence, only the intended use in EU countries belonging to the Southern European residue zone has eventually been considered by the RMS.

- 1 new trial was disregarded, due to quantifiable MITC residues found in both control and treated carrot sample. This apparent contamination was also observed in other trials conducted on other crops in the same city in Spain ([redacted]).

- In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), 4 residue trials on carrots (2 NEU, 2 SEU) had already been evaluated and accepted (BE, 2010b; EFSA, 2011). Although these trials were conducted at a higher application rate (2.4N – 2.8N compared to the representative use at 153 kg metam-sodium/ha supported by Lainco for renewal) and no quantifiable residues of either MITC or DMTU were found, these trials are no longer considered valid, due to concerns on the storage period of the samples and the stability of MITC and DMTU during storage. For further details, see **Vol.3, B.7.3.4** (Taminco – KCA 6.3.3/01).

- It should be noted that additional sets of trials (4 NEU and 6 SEU) are available on carrot. Those trials were performed in 2013-2014 with an application rate around the intended 153 kg metam-sodium/ha. They were submitted at national level after first a.s. approval and some of them (4 NEU and 4 SEU) were eventually considered during MRL review (EFSA, 2019a), where it was concluded that the 4 NEU trials (KCA 6.3.2/03) are not valid, due to concerns on the storage period of the samples. Indeed, sample storage periods were much too long (and storage conditions inappropriate), particularly considering the rapid decline of MITC and DMTU residue levels observed in storage stability investigations, so reported <LOQ results cannot be relied upon. However, taking into account new findings on storage stability (*vide supra* – **2.7.1**), also the reliability of the SEU trials (KCA 6.3.2/01 and KCA 6.3.2/02) is questionable. As a consequence, it is likely that actual residue levels in carrot roots at harvest have been underestimated by the measurements undertaken in these older trials. Still, quantifiable residue levels of MITC (up to 0.023 mg/kg) had been found in 2 of the trials conducted in SEU (in which no plastic film was used to cover treated soil) and DMTU had been found at significant levels above the LOQ in all of those SEU trials (up to 0.23 mg/kg).

  According to the applicant Lainco, those positive residue findings can be ignored due to the sample storage issue (and for some trials due to some other deviations from the representative GAP, such as the absence of soil covering with TIF). However, the RMS does not agree with the conclusions of the applicant that the positive residue findings should be ignored for these reasons; the deviating design of these trials and the identified shortcomings do not necessarily represent a more critical situation from a residue perspective; more details: see **Vol.3, B.7.3.4** – Lainco – KCA 6.3.2/01 and KCA 6.3.2/02.

  For the sake of completeness, the results from the SEU trials with positive findings – serving as relevant supplementary information – are summarised here below:

Crop (region)	***Dossier reference***	***Study report no***	***MITC (mg/kg)***	***DMTU (mg/kg)***
Carrots (SEU)	*Lainco – KCA 6.3.2/01*	*61SRES13R4*	*0.013, 0.023*	*0.063, 0.12, 0.19, 0.23*
Carrots (SEU)	*Lainco – KCA 6.3.2/02*	*61SRES14R02*	*<0.01 (4x)*	*0.062, 0.077, 0.099, 0.23*

2.7.4.5 Onions (LAINCO)

Metam formulated as Metam Sodium 51% SL is intended to be used on onion according to representative GAPs summarised in **Table 2.7.4.5-1**.

Table 2.7.4.5-1: Representative GAPs for metam on onion

Outdoor/ Greenhouse	Number of applications	Application rate	Method of application	Period of application	Remarks
Outdoor (SEU)[19]	1 application every 3 years	153 kg metam-sodium/ha	Soil injection (15-20 cm depth) in combination with the use of Total Impermeable Foil (TIF)	Spring to winter Pre-plant or pre-sowing	Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked. A cress germination test should always be performed prior to sowing or planting.

Six (6) new supervised residue trials (4 NEU, 2 SEU) were provided. The trials performed are available in support of this GAP.
The NEU trials were conducted with soil treatment (via soil injection) at an application rate ranging from 144 to 180 kg metam-sodium/ha, followed by soil coverage for 21 days (using TIF), subsequent superficial tillage, a cress test and onion transplanting 25-29 days after application. However, as only use on onions in EU countries belonging to the Southern European residue zone is supported by the applicant[19], the new NEU trials were not relied upon.
The SEU trials are considered GAP-compliant, as they were conducted with soil treatment (via soil injection) at an application rate of 148-167 kg metam-sodium/ha, followed by soil coverage for 19-21 days (using TIF), subsequent superficial tillage, a cress test and onion transplanting 25 days after application.

Analyses were performed relatively rapidly (mostly 1-3 days) after sampling. However, it should be noted that MITC residue levels in onion were found to decline significantly already beyond 1 day under frozen storage conditions (*vide supra* – **2.7.1**); on average only 55% of the fortified MITC was recovered after 2 days of frozen storage, which corresponded to 65% of the amount recovered at day-0 sampling. Therefore, for some of the trials, the reported "LOQ" residue levels for MITC in onion bulb may be an underestimation of the actual residue levels at harvest.

[19] In the applicant's dossier (Doc N2 Metam _v4.0 Oct2020), it was initially mentioned that uses on onions (and also on potatoes and carrots) are intended in the following EU Member states: BG, CY, EL, ES, FR, HR, IT, PT. As FR was included in that list, residue trials in the NEU residue zone would normally also be required to support this use in FR. However, as only new trials were provided for onion, the applicant Lainco clarified, at request of the RMS, that the addition of FR was a mistake in final dossier preparation, as it appeared there were insufficient data available to support the use in FR. As a consequence, only the intended use in EU countries belonging to the Southern European residue zone has eventually been considered by the RMS.

No quantifiable residues of MITC or DMTU were found in the onion bulbs at commercial harvest. LOQ was 0.01 mg/kg for each analyte. Results are summarised below.

Crop (region)	Dossier reference	Study report no	MITC (mg/kg)	DMTU (mg/kg)
Onions (NEU)	Lainco – KCA 6.3.3/05	RDE-19-36860	<0.01 (3x), *<0.01* †	<0.01 (4x)
Onions (SEU)	Lainco – KCA 6.3.3/04	RDE-19-38142	*<0.01 (2x)* †	<0.01 (2x)

† Despite relatively rapid analysis after sampling (2-3 days), it cannot be excluded that actual residue levels were underestimated, as MITC declined >30% beyond 1 day in onion in the storage stability study.

On the basis of these data, the applicant Lainco argues that a "no residue situation" can be concluded on for onion and that no additional data are required at least in NEU.
The applicant also announced that 6 additional residue trials on onion in the SEU residue zone (Spain, France and Italy) were planned in 2020, to complete the SEU trial data set for onions. The RMS proposes to request and assess these additional trial data during the peer review (**DATA GAP**).

- It should be noted that additional sets of trials (4 NEU and 7 SEU) are available on onion. Those trials were performed in 2013-2014 with an application rate around the intended 153 kg metam-sodium/ha, were submitted at national level after first a.s. approval and were eventually considered during MRL review (EFSA, 2019a), where it was concluded that the 4 NEU trials (KCA 6.3.3/03) are not valid, due to concerns on the storage period of the samples. Indeed, sample storage periods were much too long, particularly considering the rapid decline of MITC and DMTU residue levels observed in storage stability investigations, so reported <LOQ results cannot be relied upon.
  Based on the SEU trials (8 trials, which however included 2 replicates, so actually only 7 independent trials), an MRL of 0.15 mg/kg was derived by EFSA (EFSA, 2019a).
  However, taking into account new findings on storage stability (*vide supra* – **2.7.1**), also the reliability of these SEU trials (KCA 6.3.3/01 and KCA 6.3.3/02) is questionable, at least for MITC (and for DMTU in 4 of the trials). As a consequence, it is likely that actual residue levels in onion bulbs at harvest have been underestimated by the measurements undertaken in these older trials. Still, quantifiable residue levels of MITC (up to 0.088 mg/kg) and DMTU (up to 0.35 mg/kg) had been found in several of these SEU trials. As a consequence, the risk assessment values derived by EFSA for MITC and DMTU (see here below extracts from EFSA Reasoned Opinion – EFSA, 2019a) may need to be reconsidered as well.

MITC:

Crop	Region/ indoor[a]	Residue levels observed in the supervised residue trials relevant to the supported GAPs (mg/kg)	Recommendations/comments (OECD calculations)	MRL proposals (mg/kg)	HR (mg/kg)[b]	STMR (mg/kg)[c]
Onions	NEU	(4 × < 0.01)	Trials on onions compliant with GAP (France, 2017). Extrapolation to shallots is applicable. However, these trials are not deemed valid due to concerns on the storage period of the samples (> 3 months). No MRL can be derived from this GAP	–	–	–
	SEU	3 × < 0.01; 0.01; 0.014; < 0.016; 0.067; 0.088	Trials on onions compliant with GAP (France, 2017; Spain, 2017) MRL_{OECD} = 0.15	0.15	0.09	0.01

DMTU:

Onions	NEU	(4 × < 0.01)	Trials on onions compliant with GAP (France, 2017). Extrapolation to shallots is applicable. However, these trials are not deemed valid due to concerns on the storage period of the samples (> 3 months). No MRL can be derived from this GAP	–	–	–
	SEU	4 × < 0.01; 2 × 0.011; 0.015; 0.35	Trials on onions compliant with GAP (France, 2017; Spain, 2017) MRL_{OECD} = 0.53	0.6 (tentative)[g]	0.35	0.01

According to the applicant Lainco, the positive residue findings in the older SEU trials can be ignored due to the sample storage issue (and for some trials due to some other deviations from the representative GAP, such as the absence of soil covering with TIF). However, the RMS does not agree with the conclusions of the applicant that the positive residue findings should be ignored for these reasons; the deviating design of these trials and the identified shortcomings do not necessarily represent a more critical situation from a residue perspective; more details: see **Vol.3, B.7.3.5** – Lainco – KCA 6.3.3/01 and KCA 6.3.3/02.

- During the assessment, RMS noted that another Magnitude of Residues study in onions was mentioned in the dossier, though only in the analytical method part (dossier reference KCA 4.1.2/7 – [redacted], 2013 – study no 12056RON), and only the analytical phase report ([redacted] 2013 – 12054-06R) was included in the dossier. At request of the RMS, the applicant Lainco provided the field phase report, but clarified that the study was not deemed relevant to the residue assessment, as it does not comply with the intended GAP. Based on a quick review by the RMS, the study indeed comprised 4 trials (2 in Italy, 2 in Spain) involving (ca. 2x) higher application rates (299 – 331 kg metam-sodium/ha) compared to the intended GAP in the framework of renewal. Therefore, the positive findings for both MITC and DMTU reported in that study (MITC 0.01 – 0.016 mg/kg; DMTU 0.021 – 0.053 mg/kg) have not been considered further by the RMS.

2.7.4.6 Peppers (LAINCO)

Metam formulated as Metam Sodium 51% SL is intended to be used on pepper according to representative GAPs summarised in **Table 2.7.4.6-1**.

Table 2.7.4.6-1: Representative GAPs for metam on onion

Outdoor/ Greenhouse	Number of applications	Application rate	Method of application	Period of application	Remarks
Greenhouse[20]	1 application every 3 years	306 kg metam-sodium/ha	Drip irrigation in combination with the use of Total Impermeable Foil (TIF)	Spring to winter Pre-plant or pre-sowing	The product must be injected into the drip irrigation system undiluted. The proportion of the total water volume applied via the irrigation system should not be less than 2%. Total Impermeable Foil (TIF) must be used for at least the duration of the waiting period. After application, a waiting period of 21 days should be respected, after which the soil should be superficially re-worked. A cress germination test should always be performed prior to sowing or planting.

As pepper is a major crop, at least 4 GAP-compliant trials demonstrating a <LOQ residue situation (for both MITC and DMTU) are required, in accordance with SANCO 7525/VI/95 rev.10.3 (EC, 2017).

Fully valid and GAP-compliant residue trials in support of this use on peppers are however not available; only trials with deviating trial design (in particular absence of TIF) are available and the reliability of some results (in particular for MITC) is questionable. An overview of the available trials, which can be considered as supportive information only, is presented further below. In summary, <LOQ results for MITC were obtained in 9 trials, though with limited reliability due to the fact that no robust conclusion could be drawn on storage stability in pepper (cf. 2.7.1). DMTU was not found at or above the LOQ in any of the 8 selected trials (and in only 2 trials of those reliability of the findings is questioned due to sample storage duration). Furthermore, DMTU was <LOQ in the 2 trials with higher (ca. 2N) application rates.
Overall, RMS is of the opinion that (at least 4) fully GAP-compliant trials on pepper (with rapid analysis of MITC after sampling) are still required to confirm the <LOQ residue situation under conditions representative of the intended use (e.g. with TIF) (**DATA GAP**).

- To support this use on peppers (under protected conditions), the applicant Lainco referred to the trials previously evaluated in the framework of the previous EU peer review of metam (under Dir. 91/414/EEC). Indeed, 3 indoor trials had been considered valid under the previous peer review (cf. EFSA, 2011). Those trials were conducted in plastic tunnels and at a more critical application rate (577 – 602 kg metam-sodium/ha, i.e. almost 2N compared to the representative use supported in the framework of renewal of a.s. approval, see above), but without use of plastic film to cover the soil after treatment. Furthermore, some other experimental conditions deviated from the new intended use, e.g. in one of the trials, the waiting period between treatment and planting was more critical (14 days instead of 21 days) and the product was applied (undiluted) via soil

[20] The applicant Lainco clarified that the product is intended to be used in permanent glasshouses as well as walk-in tunnels. For the purpose of the residues assessment, this has been considered as an 'indoor' use (under protection).

injection (instead of dilution via the drip irrigation system), while in another trial, no soil working was done after application.

No quantifiable residues of MITC or DMTU were found in the pepper fruits at commercial harvest. LOQ was 0.01 mg/kg for each analyte. Results are summarised below.

Crop (region)	**Dossier reference**	**Study report no**	**MITC (mg/kg)**	**DMTU (mg/kg)**
Pepper (indoor)	*Taminco – KCA 6.3.1/02*	*08083RMC*	*<0.01 †*	-
	Taminco – KCA 6.3.3/04	*09059RGP*	*<0.01 (2x) †*	*<0.01 (2x) †*

† With regard to the reliability of these results, it should be noted that the sample storage periods were only slightly above or covered by the period of storage stability demonstrated for MITC and DMTU in pepper in the study evaluated in the framework of first EU peer review of the active substance dazomet ([redacted], 1998); see **2.7.1**. Nevertheless, taking into account the apparent discrepancy between results from different, guideline-compliant storage stability studies for another fruiting vegetable commodity (tomato) and the fast decline observed in other fruiting vegetables (e.g. max. 3 days for MITC and max. 14 days for DMTU in cucumber), the presumed stability period for MITC and DMTU in pepper is affected by an additional uncertainty. If, looking at all the results for the commodity type fruiting vegetables, a maximum storage period of 3 days (MITC) and 14 days (DMTU) would be tentatively presumed also for pepper, the reliability of these trial results could be questioned.

On the basis of these data, the applicant Lainco argued that a "zero residue situation" can be concluded on for pepper and that no additional data be required. However, according to Reg. (EU) No 283/2013, a 'zero' residue situation shall be predicted from representative plant metabolism studies and/or where no detectable residues occur in studies with exaggerated application rates. RMS is of the opinion that these criteria are not fulfilled, taking into account the quantifiable residues of MITC observed in one trial at higher application rate (see below; KCA 6.3.4/01).

However, since the influence of the method of application on the magnitude of residues available for uptake is unknown and since it cannot be excluded that use of a TIF would lead to more residues available in soil at planting for potential uptake by the crop, the residue trials cannot be considered fully GAP-compliant and are to be considered as supportive information only.

- It should however be noted that 8 additional indoor trials on pepper (performed in 2011-2012) are available; these trials were submitted at national level after first a.s. approval and have not been previously peer reviewed at EU level. In those trials, metam-sodium was applied via drip irrigation at rates in the range of 311-328 kg/ha (i.e. around the intended 306 kg metam-sodium/ha). In 4 of these trials, additional plots were also treated at a higher application rate (614-615 kg metam-sodium/ha).

Also in these trials, no TIF was used, so they cannot be regarded as fully GAP-compliant trials, as acknowledged by the applicant Lainco, only as supportive information. This was acknowledged by the applicant Lainco.

For the sake of completeness, the results from the additional indoor trials are summarised here below:

Crop (region)	***Dossier reference***	***Study report no***	***MITC (mg/kg)***	***DMTU (mg/kg)***
Pepper (indoor)	*Lainco – KCA 6.3.4/01*	*11027RGP*	*<0.01 (2x) †*	*<0.01 (2x)*
	Lainco – KCA 6.3.4/02	*12052RGP*	*<0.01 (4x) †*	*<0.01 (4x)*

† With regard to the reliability of these results, it should be noted that the sample storage periods were covered by the period of storage stability demonstrated for MITC and DMTU in pepper in the study evaluated in the framework of first EU peer review of the active substance dazomet ([redacted], 1998); see 2.7.1. Nevertheless, taking into account the apparent discrepancy between results from different, guideline-compliant storage stability studies for another fruiting vegetable commodity (tomato) and the fast decline observed in other fruiting vegetables (e.g. max. 3 days for MITC and max. 14 days for DMTU in cucumber), the presumed stability period for MITC and DMTU in pepper is affected by an additional uncertainty. If, looking at all the results for the commodity type fruiting vegetables, a maximum storage period of 3 days (MITC) and 14 days (DMTU) would be tentatively presumed also for pepper, the reliability of the <LOQ results for MITC could be questioned.

- Note: Only the results of the experiments at higher application rates (KCA 6.3.4/01) were eventually considered during MRL review (EFSA, 2019a), considering the critical EU indoor GAP (612 kg/ha) notified in that framework. Based on those 4 indoor trials, a tentative MRL of 0.03 mg/kg for MITC was calculated by EFSA; see here below extracts from EFSA Reasoned Opinion (EFSA, 2019a). Results for DMTU were not considered valid, *"due to concerns on the storage period of the samples (>1 month)"*, although according to a review of the studies by the RMS, the storage period exceeded 14 days only in 1 of those four trials.

MITC:

Sweet peppers	SEU	–	No data available	–	–	–
	Indoor	< 0.01; < 0.01; < 0.01; 0.012	Trials on peppers compliant with GAP (France, 2017) MRL_{OECD} = 0.03	0.03 (tentative)[(d),(e)]	0.01	< 0.01

DMTU:

Sweet peppers	SEU	–	No data available	–	–	–
	Indoor	(4 × < 0.01)	Trials on peppers compliant with GAP (France, 2017). However, these trials are not deemed valid due to concerns on the storage period of the samples (> 1 month). No MRL can be derived from this GAP	–	–	–

2.7.4.7 Ornamentals (TAMINCO)

No data were provided that address the magnitude of residues in ornamental crops grown in soil treated with metam(-sodium). For the purpose of consumer risk assessment and MRL setting, these data are not directly required for a non-edible crop. However, with regard to the transfer of residues to honey and bee products: see **2.7.8**.

2.7.4.8 Other (tomatoes, cucumber, aubergine)

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), also representative uses on other crops (i.e. tomatoes, cucumber and aubergine, strawberry and grapes) were assessed (cf. EFSA, 2008; EFSA, 2011). Residue trials (conducted in the EU) were provided and assessed in the original DAR (BE, 2010b) for some of those crops (tomatoes, cucumber and aubergine). These crops are not relevant for the representative uses supported in the framework of renewal of a.s. approval. Nevertheless, for the sake of completeness, the assessment of these trials – updated where necessary taking into account new conclusions on storage stability and/or analytical method validation – has been integrated in the Draft Renewal Assessment Report; see **Vol.3, B.7.3.8**.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

2.7.5.1 Animal dietary burden estimation

For the judgement on relevance of animal feed items and for the estimation of the animal dietary burden resulting from the representative uses, the following guidelines and recommendations have been applied:

- the OECD feedstuff table (EU diets for 9 different animal species) in the OECD guidance document No 73 on residue in livestock (OECD, 2013), as well as the calculation tool developed by EFSA (*Animal model 2017.xls* – EFSA, 2017)[21].
- The EU working document on the nature of pesticide residues in fish (European Commission, 31 January 2013 – SANCO/11187/2013 Rev. 3)

TAMINCO

The crops concerned by the representative uses supported by Taminco (lettuce, baby leaves and ornamentals) are not fed to poultry, ruminant, pigs or fish according to the EFSA Animal Model 2017 (or SANCO/11187/2013 rev.3 for fish). Therefore, these uses do not require any further consideration of transfer of residues from feed of plant origin to animal products.

LAINCO

Among the crops concerned by the representative uses supported by Lainco (potato, carrot, onion, pepper), potato and carrot are relevant as livestock feed items. The applicant provided two separate estimations of livestock exposure, to MITC and DMTU respectively, using input values derived from the new residue trials only and applying the tentative processing factors derived for MITC for some processed potato feed items. For DMTU, no processing factors are available, but no default processing factors were applied either by the applicant, presumably because the residues were below the LOQ in potato tubers in the new trials and residues in processed potato commodities are expected to be below the LOQ as well.
Input values proposed by the applicant are presented further below (in grey) for the sake of transparency and completeness; see **Table 2.7.5.1-1**. The applicant concluded that:

- for MITC the trigger value of 0.004 mg/kg bw is not exceeded and that therefore no livestock metabolism studies nor feeding studies be required; see **Table 2.7.5.1-2**.
- For DMTU, the calculation proposed by the applicant showed that the trigger value of 0.004 mg/kg is slightly exceeded for sheep (0.0050 mg/kg bw per day and 0.0044 mg/kg bw per day for lamb and ram/ewe respectively) with carrot culls as the highest contributor for these two diets; see **Table 2.7.5.1-3.** The applicant argued that the exceedance of the trigger is due to the input (HR) value of 0.06 mg/kg in carrot roots, which the applicant considers to be an outlier that should not be taken into account. Based on this, the applicant claimed that livestock metabolism studies or feeding studies are not required.

However, in the opinion of the RMS, the selection of the input values by the applicant is not appropriate; the datasets of valid and fully GAP-compliant trials on potato and carrots is incomplete and furthermore, there is no convincing evidence available that could justify the omission of the quantifiable residue findings in the older residue trials (see **2.7.4**). Although the magnitude of residues in possible feed items remains insufficiently clarified, the RMS has performed a **tentative** livestock dietary burden using the more critical residue input values derived from the older field trials with quantifiable (though underestimating) residue levels as surrogate levels, although it should be recalled that these were probably still underestimations.

- MITC: Using the more critical input values derived from the field trials with quantifiable (though underestimating) residue levels (carrot STMR/HR 0.01/0.023; potato STMR/HR 0.033/0.055) the trigger value of 0.004 mg/kg bw/day is slightly exceeded for some animal diets: see **Table 2.7.5.1-4**.
- DMTU: Using the more critical input values derived from the field trials with quantifiable (though underestimating) residue levels (carrot STMR/HR 0.11/0.23; potato STMR/HR 0.011/0.084), and by applying the default processing factors, the trigger value of 0.004 mg/kg bw/day is clearly exceeded for all animal diets; with carrot culls as the highest contributor; see **Table 2.7.5.1-5**.

[21] Cugier, Jean-Pierre, & Ferreira, Lucien. (2017). EU Animal burden calculator - animals. *https://zenodo.org/record/827275#.Wco5TU8Uncs*

With regard to fish, Lainco noted that, while some of the crops may be incorporated in farmed fish feed items (*i.e.* potato), it is highly unlikely that residues of metam or its metabolites will be transferred to and accumulate in fish at significant levels, because of the *"negligible residues of metam within harvestable crops"* and because of the hydrophilicity of metam-sodium, MITC and DMTU (reference to Log Pow values was made).

CONCLUSION:

RMS concludes that, on the basis of the information currently available, a livestock dietary burden above the legal trigger of 0.004 mg/kg b.w./day cannot be excluded, neither for MITC, nor for DMTU and therefore, waiving of the need for livestock metabolism studies is not accepted on this basis **(data gap;** relevant for the representative uses on potatoes and carrots).
With regard to potential residues in fish, RMS considers that no further consideration is needed, since residues of metam are not lipophilic (cf. section **2.2.1**: log Pow values for metam, MITC and DMTU are far below the trigger value of 3) and are therefore not expected to accumulate within fish tissues.

Table 2.7.5.1-1: Input values used for dietary burden calculation – proposal APPLICANT

Feed commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Risk assessment residue definition 1: MITC				
Potato, culls	0.01	STMR from new residue trials	0.015	HR from new residue trials
Potato, process waste	0.006 (0.01 x 0.6)	STMR x PF(1)	0.009 (0.015 x 0.6)	HR x PF(1)
Potato, dried pulp	0.028 (0.01 x 2.8)	STMR x PF(2)	0.042 (0.015 x 2.8)	HR x PF(2)
Carrot, culls	0.01	STMR from new residue trials	0.01	HR from new residue trials
Risk assessment residue definition 2: DMTU				
Potato, culls	0.01	STMR from new residue trials	0.01	HR from new residue trials
Carrot, culls	0.01	STMR from new residue trials	0.06	HR from new residue trials

(1) highest (tentative) PF from wet peel and dry peel; see **2.7.6.3**
(2) tentative PF for dried pulp; see **2.7.6.3**

Table 2.7.5.1-2: Results of the dietary burden calculation for MITC (considering only new trials – tentative)

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0,001	0,001	0,04	0,04	Dairy cattle	Potato	culls	No
Cattle (dairy only)	0,001	0,001	0,03	0,04	Dairy cattle	Potato	culls	No
Sheep (all diets)	0,001	0,001	0,04	0,04	Ram/Ewe	Potato	culls	No
Sheep (ewe only)	0,001	0,001	0,04	0,04	Ram/Ewe	Potato	culls	No
Swine (all diets)	0,001	0,001	0,04	0,05	Swine (finishing)	Potato	culls	No
Poultry (all diets)	0,001	0,001	0,01	0,02	Turkey	Potato	culls	No
Poultry (layer only)	0,001	0,001	0,01	0,01	Poultry layer	Carrot	culls	No

Table 2.7.5.1-3: Results of the dietary burden calculation for DMTU (considering only new trials – tentative)

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0,002	0,004	0,05	0,11	Dairy cattle	Carrot	culls	No
Cattle (dairy only)	0,002	0,004	0,04	0,10	Dairy cattle	Carrot	culls	No
Sheep (all diets)	0,002	0,005	0,05	0,13	Lamb	Carrot	culls	Yes
Sheep (ewe only)	0,002	0,0044	0,05	0,13	Ram/Ewe	Carrot	culls	Yes
Swine (all diets)	0,001	0,004	0,04	0,14	Swine (finishing)	Carrot	culls	No
Poultry (all diets)	0,001	0,004	0,01	0,05	Poultry broiler	Carrot	culls	No
Poultry (layer only)	0,001	0,003	0,01	0,05	Poultry layer	Carrot	culls	No

Table 2.7.5.1-4: Results of the dietary burden calculation for MITC (considering most critical residue levels – tentative)

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0.004	0.005	0.12	0.15	Dairy cattle	Potato	culls	Yes
Cattle (dairy only)	0.004	0.005	0.10	0.13	Dairy cattle	Potato	culls	Yes
Sheep (all diets)	0.004	0.005	0.12	0.15	Ram/Ewe	Potato	culls	Yes
Sheep (ewe only)	0.004	0.005	0.12	0.15	Ram/Ewe	Potato	culls	Yes
Swine (all diets)	0.003	0.005	0.12	0.17	Swine (finishing)	Potato	culls	Yes
Poultry (all diets)	0.003	0.004	0.04	0.06	Turkey	Potato	culls	No
Poultry (layer only)	0.002	0.003	0.03	0.04	Poultry layer	Potato	culls	No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Table 2.7.5.1-5: Results of the dietary burden calculation for DMTU (considering most critical residue levels – tentative)

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0.026	0.0322	0.87	1.02	Dairy cattle	Carrot	culls	Yes
Cattle (dairy only)	0.026	0.0322	0.69	0.84	Dairy cattle	Carrot	culls	Yes
Sheep (all diets)	0.031	0.0372	0.92	1.12	Ram/Ewe	Carrot	culls	Yes
Sheep (ewe only)	0.031	0.0372	0.92	1.12	Ram/Ewe	Carrot	culls	Yes
Swine (all diets)	0.014	0.0195	0.60	0.85	Swine (breeding)	Carrot	culls	Yes
Poultry (all diets)	0.013	0.0202	0.19	0.29	Poultry broiler	Carrot	culls	Yes
Poultry (layer only)	0.011	0.0180	0.16	0.26	Poultry layer	Carrot	culls	Yes

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

2.7.6 Summary of effects of processing

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC) studies investigating the nature and magnitude of residues in processed commodities were not provided, but these were not deemed required at that time, because neither MITC nor DMTU had been found at quantifiable levels (≥ LOQ 0.01 mg/kg) in the trials available for peer review (cf. EFSA, 2011).

However, it is noted that quantifiable residue levels of MITC and DMTU were observed in trials conducted after first approval of metam (see also **2.7.4**). Considering the significant residue levels observed in several commodities (well above 0.01 mg/kg, and in some cases >0.1 mg/kg), it was therefore concluded during the MRL review that studies investigating the behaviour of MITC and DMTU through standard hydrolysis conditions are required (EFSA, 2019a).

In the framework of renewal of approval, new data were provided by the applicant Lainco, i.e. a high temperature hydrolysis study for both MITC and DMTU, as well as information regarding the magnitude of MITC residues in processed potato commodities. Results are summarised further below.
No data were provided by the applicant Taminco, which is acceptable given the representative uses supported for renewal; the need for studies investigating the impact of processing on the nature and/or magnitude of residues has been acceptably waived by the applicant Taminco, with reference to the fact that the representative crops (lettuce, baby leaves and ornamentals), and possible edible commodities derived thereof, are not subjected to processing according to OECD Series on Testing and Assessment No. 96[22].

2.7.6.1 Nature of the residues

The stability of [thiocarbonyl-^{14}C]-MITC and [thiocarbonyl-^{14}C]-DMTU under different hydrolysis conditions representative of main processing conditions (pasteurization, baking/brewing/boiling and sterilization) was investigated in separate studies in line with OECD recommendations (TG507).

- MITC was found to be reasonably stable to hydrolysis in buffer solutions at pH 4 under conditions representative of pasteurisation. At pH 5 and pH 6, under conditions representative of backing/brewing/boiling and sterilisation respectively, MITC partially degraded to minor, unidentified degradates (all individually representing < 10% AR) and carbon dioxide (CO2). Although only 57% of the applied recovery in the experiment with sterilization could be recovered, it can be reasonably expected that the loss of radioactivity was due to the volatilization of CO_2 (formed after hydrolytical degradation of MITC), as demonstrated in the experiment simulating baking/brewing/boiling conditions (using volatiles traps). DMTU (N,N'-dimethyltiourea) was detected at low levels in the processed samples, but also in the ambient control samples. DMU (N,N'-dimethylurea) was not detected in any sample.
  RMS notes that due to the position of the radiolabel [thiocarbonyl-14C], the study did not directly provide information on all degradation products formed by hydrolysis of MITC. However, considering the relatively simple chemical structure of MITC and the confirmed formation of 14CO2, it can be reasonably expected that methylamine (CH3-NH2) and HS- or H2S would also be formed by hydrolysis.

- DMTU was found to be hydrolytically stable in buffer solutions at pH 4, pH 5 and pH 6 at temperatures which simulated pasteurisation (90°C), baking/brewing/boiling (100°C) and sterilisation (120°C) respectively.

On the basis of these findings, the RMS concludes that a specific residue definition for processed commodities is not necessary, neither for risk assessment, nor for monitoring purposes.

2.7.6.2 Distribution of the residue in edible peel and pulp

No data were provided, but no data are deemed required, taking into account the representative uses on edible crops supported for renewal of a.s. approval (i.e. lettuce, baby leaf crops, potato, carrot, onion and pepper), which do not comprise plant products with inedible peel.

[22] OECD Guidance document on magnitude of pesticide residues in processed commodities (OECD, 29 July 2008 – ENV/JM/MONO(2008)23)

2.7.6.3 Magnitude of residues in processed commodities

Potato tuber samples originating from 2 different (SEU) field residue trials containing quantifiable levels of residues of the main metabolite MITC were processed according to different processes (baking, French fries processing, canning and potato starch processing) and the MITC content was determined in the various processed potato fractions obtained. It should be noted that from the first trial, both control and treated potato samples (with MITC 1.9-2.1 mg/kg) were taken for processing, whereas from the second trial, only one (treated) potato sample was used for processing and this raw potato sample contained much lower residues, slightly above the LOQ (0.017 mg/kg).

Dilution of MITC was observed in most processed commodities, except in potato pulp (wet and dry) where a concentration of residue was obtained. Processing factors derived from this study are to be regarded as *indicative* for most of the processed potato fractions, except for wet pulp and baked potatoes. As a matter of fact, due to the low MITC content in the raw, unprocessed potato sample from the second trial, residues were <LOQ in most of the processed fractions. Hence, for most of the processed commodities, transfer factors from two independent field test sites were not available; they were derived of processing of the control and treated sample from the same trial site and in those cases, the highest transfer factor was selected.

The (*indicative*) processing factors derived for the processed potato fractions are as follows:

Wet peel: 0.1
Dry peel: 0.6
Wet pulp: 0.9; 1.2 → median 1.1
Dry pulp: 2.8
Boiled potatoes: 0.6
Microwaved potatoes: 0.9
French fries: 0.1
Baked potatoes: 0.3; 0.8 → median 0.6

2.7.7 Summary of residues in rotational crops

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC) studies investigating the nature and magnitude of residues in rotational crops were not provided, but were not deemed required for the following reasons (cf. BE, 2010b; EFSA, 2011).

- The DT_{90} in soil was less than 100 days, for both metam and its metabolite MITC. It was also demonstrated that DMTU is not persistent, as the average DT50 value of DMTU in soils is 0.24 days.
- Furthermore, taking the use pattern – as a soil fumigant before the crop is planted – into account, the primary plant metabolism data also covers rotational crops; residues in rotational crops would not be expected to be qualitatively different compared to those in primary crops.

In the framework of renewal of approval, it was confirmed that metam, MITC and DMTU are degraded rapidly in soil (with DT_{90} values well below 100 days) – see **2.8.1** – and therefore, no additional studies were provided and are still not required.

2.7.8 Summary of other studies

2.7.8.1 Effect on the residue level in pollen and bee products

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), no data were provided to address the possibility of residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom; this was not yet a legal data requirement at that time.

In the framework of renewal of approval, no new additional experimental data were provided to address the new legal data requirement (cf. Reg. (EU) No 283/2013); both applicants provided waivers, which are presented here below for the sake of completeness.

TAMINCO:

<<

No additional data are provided in support of this application.

Generally, for greenhouse uses, it can be reasonably assumed that only few bees, if any, are exposed to potential residues in pollen and nectar, and the overall contribution to honey can be considered negligible.

Taking that aside, a study for determining the magnitude of metam residues in honey is not required for the following reasons.

According to SANTE/11956/2016 rev. 9 Appendix II (14 September 2018, implemented by 01.01.2020), the representative uses lettuce and baby leaf crops have no melliferous capacity, while flowering ornamentals do have melliferous capacity. However, metam is a soil fumigant applied before planting or sowing, i.e. not during the flowering stage. Moreover, metam in not systemic based on metabolism studies in plants where radioactivity had been metabolized extensively and to a large extent incorporated into natural compounds; neither parent nor any metabolites were identified.

Furthermore, application occurs six weeks before planting or sowing of the target crops with subsequent soil sealing by totally impermeable film (TIF), thus preventing any bees to access any potentially flowering weeds still present after application. As metam acts as an herbicide, no flowering weeds are expected to be present after removal of the TIF.

Regarding rotational crops, it should be noted that metam, MITC and DMTU are not persistent, and given that the use pattern of metam is a soil fumigation before planting, the primary plant metabolism studies cover the metabolism of metam in rotational crops, i.e. showing that the compound is not systemic. Therefore, no residues of metam or any metabolites are expected in pollen / nectar from rotational crops, either.

>>

LAINCO:

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Considering the representative uses (soil application before sowing/planting) and the residue levels observed in residue trials, studies on the effect on the residue level in pollen and bee products are not required.

>>

Overall, **RMS** concludes that possible transfer of residues from crops into honey (and pollen and other bee products) is irrelevant for the following representative uses supported in the framework of renewal of approval:

- Lettuce (not melliferous)
- Baby leaf crops (not melliferous)
- Potato (not melliferous*)
- Carrot (not melliferous*)
- Onion (not melliferous*)

[*RMS assumes that the GAPs as notified by the applicant do not cover the particular cultivation of the crop for seed production.]

However, for the representative uses on the following (melliferous) crops, further consideration is required.

- Ornamentals (greenhouse)
- Pepper (greenhouse)

No data are available on the nature and magnitude of residues in the aerial parts of ornamental crops. However, residues of MITC and DMTU were <LOQ in leaves of lettuce grown under protection in soil treated at the same application rate as intended for ornamentals (*vide supra* – **2.7.4.1**). Therefore, it is reasonable to deduce that residues in pollen and/or nectar from ornamental crops are expected to be negligible with respect to possible transfer of residues to honey and consumption by humans.

With regard to peppers, although supportive data provisionally suggest that residues of MITC and DMTU will be absent or be present at low levels in pepper fruits, fully valid and GAP-compliant residue trials are not available (*vide supra* – **2.7.4.6**) and it is noted that significant residues of MITC (up to 0.34 mg/kg) were found in trials on another fruiting vegetable, i.e. cucumber grown indoor in soil treated at about the same application rate as intended for pepper (cf. EFSA, 2019a). Also DMTU was found in those cucumber trials (up to 0.069 mg/kg in a trial at 1.5N rate). Therefore, the low residue situation claimed for the use (rate) on pepper cannot be regarded as sufficiently supported by experimental data and the nature and magnitude of residues in pollen and/or nectar from pepper flowers remains uncertain (**data gap**).

2.7.9 Estimation of the potential and actual exposure through diet and other sources

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), the acute and chronic consumer dietary intake of residues of MITC, resulting from the representative uses of metam supported at that time, had been estimated according to the internationally agreed methodology using the consumption data included in revision 2 of the EFSA Pesticide Residue Intake Model (PRIMo[23]). Assuming residues of MITC at maximum 0.01 mg/kg in those crops, the exposure was estimated to correspond to max. 2.5% of the ADI for MITC (0.004 mg/kg bw/day) and to max. 5% of the ARfD for MITC (0.03 mg/kg bw); see previous EFSA conclusion (EFSA, 2011).

It is noted that in the framework of the MRL review, two separate indicative consumer exposure calculations were performed using PRIMo rev.2, one for MITC, but also one for DMTU. In that framework, for the purpose of assessing the risk of dietary exposure to residues of DMTU, the toxicological reference values derived for metam were used, i.e. an ADI of 0.001 mg/kg bw/day and an ARfD of 0.1 mg/kg bw (EFSA, 2019a).

In the framework of renewal of a.s. approval, the acute and chronic consumer dietary risks resulting from the representative uses were calculated according to the internationally agreed methodology using the consumption data included in revision 3.1 of the EFSA Pesticide Residue Intake Model (PRIMo[24]).
In **Table 2.7.9-1**, an overview is presented of the toxicological reference values that have been applied to the different components included in the residue definitions for risk assessment.

Table 2.7.9-1: Toxicological reference values used for consumer risk assessment

Residue definition for risk assessment	ADI [mg/kg bw/d]	ARfD [mg/kg bw]	Remark
MITC	0.004	0.03	No change to the toxicological reference values of MITC is proposed (compared to the ADI and ARfD established in the framework of the previous EU review of metam; EFSA, 2011) (*vide supra* – ***2.6.11*** *and* ***2.6.12***).
DMTU	0.004 (tentative)	0.03 (tentative)	It is acknowledged that in previous evaluation frameworks (EFSA, 2011; EFSA, 2019a) the toxicity of metabolite DMTU was considered equivalent to the toxicity of the parent compound metam (ADI 0.001 mg/kg bw/day; ARfD 0.1 mg/kg bw). However, following reconsideration in the framework of the renewal of a.s. approval, the RMS is of the opinion that it be more appropriate to apply tentatively the toxicological reference values derived for MITC, because this is more conservative as regards ARfD, and because limited toxicological data for DMTU are available (*vide supra* – ***2.6.11*** *and* ***2.6.12***).

[23] EFSA (2007). PRIMo rev.2 – Pesticide Residue Intake Model. http://doi.org/10.5281/zenodo.56287. See also EFSA (2007). Reasoned opinion on the potential chronic and acute risk to consumers health arising from proposed temporary EU MRLs. 15 March 2007. *Available online:* www.efsa.europa.eu

[24] EFSA (European Food Safety Authority), 2019. Pesticide Residues Intake Model for assessment of acute and chronic consumer exposure to pesticide residues-rev.3.1.
Available from http://www.efsa.europa.eu/en/applications/pesticides/tools

Taking the data gaps on the magnitude of residues into account (see **2.7.4**), only a tentative consumer exposure assessment could be performed; except for lettuce and baby leaf crops, only tentative input values could be derived: see **Table 2.7.9-2**.
The outcome of the tentative consumer dietary risk assessment is summarised in **Table 2.7.9-3**.
According to the tentative calculation, the International Estimated Daily Intake (**IEDI**) of MITC related to the representative uses accounted for **max. 5% of the ADI** (PT general), with potato products showing the highest contribution to the chronic intake (up to 4% of ADI). The International Estimated Short-Term Intake **(IESTI)** of MITC was below the ARfD for all commodities concerned (max. 28% ARfD; potatoes – UK infant). Specifically for processed commodities, no exceedance of the ARfD was identified either. For DMTU, the highest calculated IEDI represents 5% of the tentative ADI and the highest calculated IESTI corresponds to 49% of the tentative ARfD (short-term intake of carrots by UK infant). The tentative dietary risk assessment for the representative uses does not reveal a chronic or an acute risk for the consumer, but it should be noted that the results are to be regarded as preliminary, taking into account the remaining uncertainty on the magnitude of residues in commodities at harvest. **The consumer risk assessment could not be finalized (DATA GAP)**.

Table 2.7.9-2: Input values for the TENTATIVE consumer dietary exposure assessments

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
MITC				
Potato	0.033	STMR (tentative)[a]	0.055	HR (tentative)[a]
Potatoes, fried	-	-	0.0055	HR (tentative)[a] x PF (tentative)[b]
Carrot	0.01	STMR (tentative)[a]	0.023	HR (tentative)[a]
Onion	0.014	STMR (tentative)[a]	0.088	HR (tentative)[a]
Pepper	0.01	STMR (tentative)[a]	0.01	HR (tentative)[a]
Lettuce	0.01	STMR (=LOQ)	0.01	HR (=LOQ)
Baby leaf crops[c]	0.01	STMR (=LOQ)	0.01	HR (=LOQ)
DMTU				
Potato	0.011	STMR (tentative)[a]	0.084	HR (tentative)[a]
Carrot	0.11	STMR (tentative)[a]	0.23	HR (tentative)[a]
Onion	0.011	STMR (tentative)[a]	0.35	HR (tentative)[a]
Pepper	0.01	STMR (tentative)[a]	0.01	HR (tentative)[a]
lettuce	0.01	STMR (=LOQ)	0.01	HR (=LOQ)
Baby leaf crops[c]	0.01	STMR (=LOQ)	0.01	HR (=LOQ)

[a] input values for potato, carrot, onion and pepper are tentative; they correspond to the most critical quantifiable residue levels found in the older supervised residue trials, for which no convincing evidence is available that could justify the omission of those quantifiable residue findings, for which it is noted that they were likely even underestimations of the actual levels; see **2.7.4**.
[b] For the specific acute risk assessment related to the consumption data for processed commodities, the same residue input values (expressed on Raw Agriculture Commodity basis) as for the overall dietary risk assessment were used, except for fried potatoes, for which the tentative processing factor of 0.1 for MITC (for French fries; see **2.7.6.3**) was applied as a refinement.
[c] RMS notes that no consumption data for baby leaf crops are included in PRIMo rev.3.1. Hence, exposure of the consumer to MITC or DMTU due to consumption of baby leaf crops could not be calculated.

Table 2.7.9-3: Summary of the outcome of the TENTATIVE consumer dietary risk assessment

RD$_{RA}$	Analyte(s) considered for dietary exposure	Chronic risk			Acute risk	
		TMDI [max. % ADI] (MS diet)	IEDI [max. %ADI] (MS diet)	Highest contributing commodity to exposure	IESTI [max. % ARfD]	Commodity with highest % ARfD (MS diet)
(1)	MITC	n.a.	5 (PT general)	Potatoes	28	Potatoes (UK infant)
(2)	DMTU	n.a.	5 (DK child)	Carrots	49	Carrots (UK infant)

2.7.10 Proposed MRLs and compliance with existing MRLs

In the framework of the previous EU peer review of metam (under Dir. 91/414/EEC), it was provisionally concluded, on the basis of the limited data available at that time, that an MRL of 0.01* mg/kg (MITC) was appropriate for all representative crops (EFSA, 2011). However, EU MRLs were temporarily established in Annex IIIA of Reg. (EC) No 396/2005, at 0.02* mg/kg for methyl isothiocyanate (MITC) (cf. Reg. (EC) No 149/2008). In 2016, several EU MRLs for MITC (incl. for lettuces, baby leaf crops and sweet peppers) were increased, to accommodate uses of dazomet, another MITC-generating active substance (cf. Reg. (EU) No 2016/1).

All existing EU MRLs for both metam and dazomet were fully reviewed by EFSA in the framework of art.12 of Reg. (EC) No 396/2005 (EFSA, 2019a; EFSA, 2019b), which led to the agreement by the European Commission and Member Statement (at the SCoPAFF Residues meeting of February 2021) on revised EU MRLs for MITC for inclusion in Annex II of Reg. (EC) No 396/2005. At the moment of finalizing the DRAR, the regulation establishing these agreed revised EU MRLs had not yet been published.

For the crops under consideration in this assessment, i.e. those related to the representative uses supported in the framework of renewal of approval of the active substance metam, both applicants claimed that the corresponding MRLs do not need to be modified.

- For lettuces and baby leaf crops cultivated under protection (representative uses supported by Taminco), sufficient fully GAP-compliant trials were indeed available to demonstrate that residues of MITC are <0.01 mg/kg (see **2.7.4**). Therefore the submitted data do not indicate that the existing MRLs (of 0.03 mg/kg) for MITC in these crops (which refers to a use of dazomet) needs to be modified.
- For the representative edible crops supported by Lainco (i.e. potato, carrot, onion, pepper), however, insufficient valid and fully GAP-compliant residue trials are available to derive MRL proposals (see **data gaps** identified in section **2.7.4**).

The MRLs derived – if possible – by the RMS within the framework of this assessment are presented below in **Table 2.7.10-1**, and have been compared with currently existing EU MRLs, as well as with revised EU MRLs agreed following MRL review (but not yet applicable at the time of finalizing the DRAR).

Table 2.7.10-1: MRL recommendations

Code[a]	Commodity	Existing EU MRL[b] (mg/kg)	Proposed EU MRL (mg/kg)	Comment/justification
Enforcement residue definition: Methyl isothiocyanate (MITC) (resulting from the use of dazomet or metam)				
Representative uses				
0211000	Potatoes	0.02*/0.01*	**No MRL proposal**	The submitted data are insufficient to derive an MRL proposal for the SEU use.
0213020	Carrot	0.02/0.02	**No MRL proposal**	The submitted data are insufficient to derive an MRL proposal for the SEU use.
0220020	Onion	0.02*/0.15	**No MRL proposal**	The submitted data are insufficient to derive an MRL proposal for the SEU use.
0231020	Pepper	0.1/0.1	**No MRL proposal**	The submitted data are insufficient to derive an MRL proposal for the indoor use.
0251020	Lettuces	0.03/0.03	No change	The submitted data are sufficient to derive an MRL proposal for the indoor use (0.01* mg/kg). The submitted data do not provide evidence that the existing MRL has to be modified. Risk for consumers unlikely.
0251080	Baby leaf crops (including brassica species)	0.03/0.03	No change	The submitted data are sufficient to derive an MRL proposal for the indoor use (0.01* mg/kg). The submitted data do not provide evidence that the existing MRL has to be modified. Risk for consumers unlikely.
1040000	Honey and other apiculture products	0.05*/0.05*	No change	No evidence that the existing MRL has to be modified, but data are missing to conclude this for the representative use on peppers (**data gap**).

2.7.11 Proposed import tolerances and compliance with existing import tolerances

not applicable

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

The environmental fate properties assessment for metam is based on the Draft Assessment Report (2007-2010), the Addendum to the Draft Assessment Report (2015), the EFSA Conclusion (EFSA Journal 2011;9(9):2334) and the studies submitted for the renewal of the active substance (DRAR 2021).

All the studies on the fate and behaviour of metam in the environment were performed under GLP and according to EPA, OECD or equivalent guidelines.

A literature search (Wagner *et al.*, 2020) was carried out for the active substance metam including its variants metam-sodium and metam-potassium, its main metabolite MITC (methyl isothiocyanate) and its relevant impurity DMTU (N,N´-dimethylthiourea), as specified in Article 8(5) of Regulation (EC) No 1107/2009. The search and review itself was in accordance with the EFSA Guidance document as published in EFSA Journal 2011;9(2):2092.
The results of the literature search, following the principles of the EFSA Guidance Document entitled "Submission of scientific peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA Journal 2011;9(2):2092), are presented in detail in the Vol. 3 CA B.8, Section B.8.6.1. All details about the protocol used, the selection of databases and the list of keywords are given in Vol. 3 CA B.8 and its Appendix. The literature articles listed in the Vol. 3 CA B.8 were useful as background information but not relevant for the risk assessment in the Fate and behavior section.

2.8.1 Summary of fate and behaviour in soil

Degradation in soil

Metam

The objective of the study by [redacted] (2004) was to investigate the degradation of metam-sodium in four soils: two soils of a sandy loam/loamy sand texture (pH 5.4 and 5.7, OM 6.97 and 1.87%), a silt loam (pH 7.1, OM 7.31%) and a clay loam (pH 7.7, OM 2.89%). The procedures employed, as a consequence of the expected rapid degradation of [^{14}C]-metam-sodium, were not designed to investigate the route of degradation nor to maintain mass balance.
Samples of each soil (50 g oven dry weight equivalent) were treated with [^{14}C]-metam-sodium at a nominal application rate of 306 g a.s./m³ (15.3 mg per test sample) and incubated for up to 120 mins. At intervals throughout the incubation period (at zero time, 10, 20, 30, 45, 60, 90 and 120 min) samples were taken for analysis.

Extractable residues ranged from ca 42 - 93% for all soils. No time dependent extractability was obvious except for the clay loam soil where extractability decreased from ca 93% immediately post-application to 47% after 90 min incubation.

HPLC analysis demonstrated a rapid degradation of [^{14}C]-metam-sodium in all soil types and at both study temperatures. The amount of [^{14}C]-metam-sodium in soil extracts decreased from 55.1% at zero time to non-detectable amounts from 90 minutes onwards in sandy loam soil 1 (20 °C), from 30.7% at zero time to non-detectable amounts from 20 minutes onwards in sandy loam soil 2, from 67.7% at zero time to non-detectable amounts from 90 minutes onwards in soil silt loam, from 85.6% at zero time to non-detectable amounts from 90 minutes onwards in soil clay loam soil and from 49.1% at zero time to non-detectable amounts at 120 minutes sandy loam soil 1 (10 °C). Although this study was not designed to investigate the route of degradation of [^{14}C]-metam-sodium, it was obvious from the chromatographic data generated that [^{14}C]-MITC was the principal degradation product, formed rapidly in all soil groups.

In conclusion metam-sodium was degraded in four fresh field soils, of differing characteristics, maintained under aerobic conditions at a nominal temperature of 20 °C. DT_{50} and DT_{90} values ranged from 4 to 17 min (arithmetic mean = 10 min, geomean = 9 min, n = 4) and from 13 to 57 min (arithmetic mean = 34 min, geomean = 29.9 min, n = 4), respectively. In one soil (sandy loam) incubated at a nominal temperature of 10 ºC, the DT_{50} value was 22 min.

MITC

The second objective of the study by [redacted] (2004) was to investigate the aerobic degradation of [^{14}C]-MITC on a longer time scale (21 days), in four fresh field soils at a nominal temperature of 20 °C and one soil at 10 °C at 50% of the maximum water holding capacity in the laboratory in the dark. The study was conducted using two soils of a sandy loam/loamy sand texture (pH 5.2 and 4.5, OM 1.5 and 12.4%), a silt loam (pH 6.1, OM 6.5%) and a clay loam (pH 7.6, OM 2.4%).

Samples of each soil (50 g oven dry weight equivalent) were treated with [^{14}C]-MITC at a nominal application

rate of 43.3 g a.s./m³ (2.17 mg per test sample). The samples were incubated in Erlenmeyer flasks for up to 21 days. Duplicate incubation flasks were analysed at zero time (immediately following treatment), 1, 7 and 21 days post application. In addition a single application flask was analysed 6 h, 2, 4, 10 and 15 days post application. The overall recovery of applied radioactivity (mass balance) was quantitative for all soil samples incubated at a nominal temperature of 20 °C ranging from 88 to 103%; for the majority of samples the mass balance was in the range of 90 to 100%. For the sandy loam soil incubated at the nominal 10 °C temperature, mass balance declined to <90% after Day 7 and thereafter continued to decline to 69% by Day 21. The apparent loss of radioactivity is thought to result from leakages caused by contraction of the steel needles; there appeared to be a relationship between the extent of mineralisation and the decline in mass balance.

The distribution pattern of radioactivity, with time, was similar for all four soils incubated at a nominal temperature of 20 °C. Immediately following application, the majority of the applied radioactivity was present in the soil ethyl acetate extract (ca 93 – ca 96%) with the majority of the remainder of the mass balance being recovered in the water extract. At 6 h, the first sampling interval at which the sample headspace was purged, a major proportion of the applied radioactivity was collected in the ethyl acetate traps (ca 62 – ca 89%); levels of applied radioactivity remaining in the ethyl acetate soil extract ranged from ca 4% to ca 23%; levels remaining in the water extract were low (<3% applied). At 6 h post-application, the radioactivity recovered in the sodium hydroxide traps accounted for ca 2% - ca 8% of the applied. As incubation progressed, the proportions of the applied radioactivity accounted for by sodium hydroxide traps (subsequently shown to be attributable to [^{14}C]-CO_2) and the non-extractable residues increased. At the final sampling occasion, Day 21, these two fractions accounted for the majority of the applied radioactivity: sodium hydroxide traps ([^{14}C]-CO_2), ca 46 – ca 86% applied; non-extractable residues ca 8% - ca 38% applied, equivalent to ca 4.30 - 16.77 mg equiv./kg. Evidence that the radioactive residue was available for mineralisation was obtained during this study by continued incubation of contingency samples.

With the sandy loam soil incubated at a nominal temperature of 10 °C, the general pattern of radioactivity distribution was similar to that observed at the higher temperature. However the rate of dissipation of radioactivity from the ethyl acetate soil extracts into the ethyl acetate traps and the eventual mineralisation to $^{14}CO_2$ (as determined by the radioactivity collected by sodium hydroxide) was slower and less extensive (approx. 36% of applied radioactivity at Day 21). The overall extent of the non-extractable residues was similar at both temperatures.

The highest non-extractable residues were observed with the silt and clay loam soils. In general residues were difficult to reduce by conventional extraction techniques and consequently one residue from the silt and clay loam incubation groups was subject to organic matter fractionation. The results indicated that the majority of the radioactive residue was associated with the insoluble humin fraction.

Chromatographic analysis demonstrated that the only significant radiolabelled component present in all samples analysed was [^{14}C]-MITC. The amount of [^{14}C]-MITC in soil extracts decreased from 93.9% at zero time to non-detectable amounts from Day 15 onwards in sandy loam 2 soil (20 °C), from 97.8% at zero time to non-detectable amounts from Day 15 onwards in sandy loam 1 soil, from 99.6% at zero time to non-detectable amounts from Day 4 onwards in soil silt loam, from 95.7% at zero time to non-detectable amounts from Day 10 onwards in soil clay loam soil and from 90.9% at zero time to 15.4% of applied at Day 21 in sandy loam 2 soil (10 °C).

The rate of degradation of [^{14}C]-MITC was estimated by calculation of DT_{50} and DT_{90} values ([redacted], 2010b) using ModelMaker 4.0 and according to FOCUS kinetics guidance (FOCUS, 2006). Calculations were made in all four soils using single first-order (SFO) kinetics. With an application rate up to 153 kg/ha of metam sodium, persistence DT_{50} values ranged from 1.08 to 3.24 days and the DT_{90} values ranged from 3.60 to 10.78 days. All soils were tested at 20°C and above optimal (pF2) soil moisture, with good SFO kinetics, therefore the persistence endpoints did not require any correction for use in modelling. In one soil (sandy loam) incubated at a nominal temperature of 10 °C, the DT_{50} and DT_{90} values were 8.31 and 27.61 days, respectively.

$^{14}CO_2$ was the only significant degradation product of [^{14}C]-MITC. It was rapidly and extensively formed in all four soil types at both temperatures. Following application of [^{14}C]-MITC, non-extractable residues remained relatively low in sandy loam soils (maximum of 9.88 and 18.06%), at both incubation temperatures, over the 21 day incubation period. Non-extractable residues in silt and clay loam soils amounted up to 20.69 and 38.38%, respectively.

The objective of the study by [redacted] (2014) was to investigate the rate of degradation of [^{14}C]MITC in four fresh field soils in the dark in the laboratory at a nominal temperature of 20 ºC and at 50% maximum water holding capacity for up to 22 d. The extent of mineralisation of [^{14}C]MITC to $^{14}CO_2$ and the formation of non-extractable residues was also investigated.

Samples of four fresh field soils were treated with [^{14}C]MITC at nominal application rates of 115.5 g/m3 and 173.2 g/m3. The soils were identified as S777 (clay loam/silt loam/loam, pH 5.4, OC: 6.9%), S778 (sandy loam, pH 4.1, OC: 6.6%), S779 (sandy loam, pH 4.7, OC: 1.1%) and S780 (loam/clay loam, pH 5.9, OC: 1.7%). The study was conducted as a series of separate incubations for each soil type and treatment rate and groups were staggered over a period of time.

At regular intervals throughout incubation, duplicate samples were taken for analysis at the following time points: Zero time (immediately following test item application), 6 h, 1, 2, 4, 7, 15 and 21 days. For soil S777 (115.5 g/m³) the Day 7 samples were removed and analysed on Day 8 and for soil S780 (115.5g/m³) the Day 21 samples were

removed and analysed on Day 22.

The main degradation product was identified as $^{14}CO_2$. It was rapidly and extensively formed in all four soil types at both application rates. Following application of [^{14}C]MITC, non-extractable residues remained relatively low in all the soils, at both application rates, over the 21/22 day incubation period.

The $DegT_{50}$ and $DegT_{90}$ values were calculated for all soils at each application rate using non-linear regression of the percent parent material remaining versus time using CAKE software. The rate of degradation of [^{14}C]MITC was rapid in all soil types and at both application rates. At 20°C, DT_{50} values were in the range 1.2 – 5.0 d (geomean = 3.2 d, n = 4) for the 115.5 g/m^3 application rate and 1.6 – 7.6 d for the 173.2 g/m^3 application rate.

DMTU

[redacted] (2010) studied the degradation of [^{14}C]-DMTU in four soils (Am Fischteich (soil I), Grossstorkwitz (soil II), Speyer 2.3 (soil III) and Speyer 6S (soil IV)) following treatment at a rate of about 5 kg test item/ha and incubation at 20°C and 50% MWHC for 60 days for all soils and up to 145 days for soils I and IV.

The test item was then applied to 50 g soil samples at a target concentration of about 1.94 to 2.77 mg a.i./ kg dry soil. This target rate corresponds to an application rate of 5 kg a.i./ha assuming an even distribution of the test item in the top 20 cm soil layer and soil bulk densities of 0.9 to 1.3 g/cm^3.

The treated soil samples were incubated at 20 ± 2°C and 50% MWHC in the dark under continuous ventilation with moistened air. The exiting air was passed through a trapping system consisting of flasks of sodium hydroxide and ethylene glycol (EG) in series. Prior to treatment and at the end of the incubation period, the microbial biomass was determined for each soil. The results showed that the soils were viable during the study.

Duplicate samples (A and B) treated with the test item were taken immediately from each soil after treatment (Day 0) and after 2, 4, 12, 24 hours and after 3, 7, 14, 35 and 60 days and, additionally for soils I and IV, after 145 days. Each sample was submitted to extraction at room temperature using acetonitrile/water (4:1; v/v) followed by Soxhlet extraction using the same solvent mixture. The individual extracts were combined and concentrated. The samples were then analysed for the test item and degradation products by HPLC and/or TLC.

Mean recoveries of radioactivity during the 60-day incubation period were 94.4 ± 1.2%, 94.8 ± 2.5%, 93.8 ± 2.1% and 95.6 ± 2.5% of the applied amount for soils Am Fischteich, Grossstorkwitz, Speyer 2.3 and Speyer 6S, respectively.

Immediately after treatment (Day 0), 83.7%, 90.3%, 92.2% and 84.6% of the applied radioactivity could be extracted from soils I to IV, respectively. The total amount of extractable radioactivity decreased with time in all four soils. The decrease was fast within the first seven days and continued thereafter at a slower rate until the end of incubation. After seven days 23.4%, 21.9%, 29.4% and 16.7% of the amount applied could be extracted. By Day 60, about half of this amount (except for soil IV) was extractable i.e. 11.9%, 11.8%, 16.7% and 12.8% for soils I to IV, respectively. Samples for soils I and IV were incubated for 145 days and at that time point 10.6% and 9.5% of the amount applied was extractable.

The amount of non-extractable radioactivity was significant, reaching maximum values of 42.2%, 19.2%, 23.0% and 38.9% within 14 days, for soils Am Fischteich, Grossstorkwitz, Speyer 2.3 and Speyer 6S, respectively. At Day 60, the corresponding values amounted to 35.9%, 18.2%, 16.5% and 30.0% of the applied radioactivity. For soils I and IV, 34.8% and 32.1% remained non-extracted after 145 days of incubation.

The remaining non-extractable radioactivity from the Day 14 soil samples were submitted to harsh extractions under reflux conditions. The radioactivity extracted amounted to 9.4%, 4.1%, 4.9% and 12.4% of the amount applied for soils I, II, III and IV, respectively. Subsequent fractionation of the soil organic matter into humic acids, fulvic acids and humin fractions showed an equal distribution of the non-extractable radioactivity between mobile (fulvic acids) and the immobile fractions (humic acids and humin fraction).

The radioactivity associated with the fulvic acids was in the range 17.7%, 5.1%, 8.6% and 15.8% AR in the four soils. The humic acids and humin fractions recovered in total 15.1%, 10.0%, 9.5% and 10.7% applied radioactivity for soils I, II, III and IV, respectively.

The mineralization of [^{14}C]-DMTU increased continuously, with $^{14}CO_2$ reaching maximum mean levels of 46.3%, 64.5%, 57.9% and 52.2% of the applied radioactivity after 60 days for soils I to IV. The concentration of other volatile products was low, with maximum amounts of 0.8% of the applied radioactivity during the entire incubation period.
[^{14}C]-DMTU disappeared rapidly in all four soils. At the first sampling interval, Day 0, DMTU represented 55.6%, 65.5%, 77.4% and 67.7% of the applied radioactivity for soils I to IV, respectively. After 12 hours of incubation, it was 7.1%, 25.3%, 24.4% and 8.5% of the applied radioactivity in the four soils. After 24 hours, [^{14}C]-DMTU further decreased to 3.0%, 6.2%, 8.8% and 0.9% for soils I, II, III and IV, respectively. At later intervals no [^{14}C]-DMTU was detected anymore.

Up to ten radioactive fractions were formed of which the major ones were M1 (identified as dimethyl urea), M3 (identified as N,N'-dimethyl-2-(3-methyl-ureido)-acetamide) and M4 (identified as N,N'-dimethyl-2-(3-methyl-

thioureido)-acetamide) with the following maximum mean percentages of applied radioactivity: M1 achieved 47.5% at Day 1 in soil II, M3 achieved 11.3% at Day 14 in soil III and M4 achieved 15.4% at Day 3 in soil I. All other radioactive fractions were either transient or did not exceed 4.6% of the applied radioactivity on two consecutive sampling intervals.

Modelling DT_{50} and DT_{90} were determined in [redacted] (2010a) using ModelMaker 4.0, according to FOCUS kinetics. Modelling DT_{50} ranged from 0.080 to 0.190 days (geomean 0.121 d, n = 4). Modelling DT_{90} were in the range 0.265 – 0.632 days (geomean 0.401 d, n = 4).

The degradation of [^{14}C]-DMTU was studied in one soil according to OECD 307 ([redacted], 2011). The soil was incubated under aerobic conditions at 20°C for 86 days in dark laboratory conditions at a soil water content corresponding to pF2. Treatments were applied at a nominal dose of 2.5 mg/kg.

Total mass balance ranged from 96.0-103.4% AR over the course of the study. The main degradation product was CO_2, which accounted for a mean of 57.1% AR after 86 days. Non-extracted residues accounted for a maximum of 19.7% AR on day 48. Characterisation of non-extracted residue found that it was a mixture of fulvic and humic acids, with a smaller proportion of humin.

Major metabolites were DMU (max 39.4% AR at day 1), unknown polar compound U1 (max 11.8% AR at day 1) and unknown compound U3 (max 17.7% AR at day 7). These metabolites declined to 5.3, 1.6 and 0.6 % AR, respectively, by the end of the test at day 86. These metabolites are not considered relevant, since DMTU is a minor impurity and its metabolites will not form in large amounts relative to the active substance.

Degradation kinetics were recalculated by the Applicant as the study did not follow FOCUS kinetics guidance with respect to the initial parent concentrations. Degradation was biphasic and best described by DFOP kinetics, with a DT_{50} of 7.39 hours and a DT_{90} of 925 hours. For modelling purposes, a DT_{50} of 45.2 hours was calculated from the FOMC DT_{90} / 3.32.

The degradation of non-labelled DMTU was studied in three soils according to OECD 307 ([redacted], 2011). The soil was incubated under aerobic conditions at 20°C for 168 hours in dark laboratory conditions at a soil water content corresponding to pF2. Treatments were applied at a nominal dose of 2.5 mg/kg.

The procedural recovery results lay within the target acceptable range of 70 to 110%, showing the methodology to be working within its requirements for each batch of samples analysed. The method was validated with an LOQ of 0.05 mg/kg.

Degradation was estimated using SFO kinetics, with a DT_{50} of 2.0-5.6 h and a DT_{90} of 6.8-18.7 h.

Adsorption/desorption

Metam

The retention behaviour of metam-sodium was investigated on a cyano HPLC-column using a citrate (pH 4) or boric acid/potassium chloride (pH 9) buffered test system ([redacted], 2002a).

The capacity factor (k) of metam-sodium was calculated based on its retention time and the dead time. In order to correlate the capacity factor of metam-sodium with its K_{OC}, six reference compounds (acetanilide, atrazine, triadimenol, naphthalene, pyrazophos and DDT) with log K_{OC} values between 1.25 and 5.63 were selected and injected in the HPLC system as well. Capacity factors (k) were determined for each reference item to establish a linear calibration plot for determined log k values vs. known log K_{OC} values.

The adsorption coefficient K_{OC} of metam-sodium was determined as < 17.8 mL/g at pH 4 and 9.

The adsorption coefficient of metam sodium (74.1% purity) was investigated using HPLC, following OECD method 121 (Sidney, 2009). Since metam sodium is an ionisable substance, separate tests were conducted at each of pH 4, 7 and 9.

For each test, a solution of metam sodium (76 mg/L in HPLC mobile phase) was prepared and chromatographed alongside the reference substance acetanilide, which has a known $\log_{10}K_{oc}$ value of 1.3.

At each pH value investigated, the retention time of metam sodium (pure grade) was shorter than that of acetanilide, the lowest K_{oc} reference standard recommended by the test guideline, indicating $\log_{10}K_{oc}$ values of less than 1.3 (K_{oc} < 20).

MITC

The adsorption/desorption behaviour of ^{14}C-methyl isothiocyanate (MITC) was determined on five soils ([redacted], 2009) in the dark in the laboratory at 20 ± 2°C using the direct method according to OECD Guideline No. 106. Two soils presented a loamy sand texture (pH 5.41 and 5.55, OM 4.90 and 1.76%), one soil a clay loam

texture (pH 5.61, OM 6.95%) one soil a loam texture (pH 4.78, OM 5.67%) and the last soil had a clay texture (pH 6.86, OM 4.72%).

Based on the results obtained in preliminary tests, the adsorption phase of the main test was carried out using sterilised, air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solutions with a soil-to-solution ratio of 1:1 and 16 hours of agitation. MITC was applied at five different initial test item concentrations (50, 25, 5.0, 1.0 and 0.5 mg/L) covering two orders of magnitude. Due to the fact that equilibrium was not reached within the 48 h incubation time of the desorption kinetics experiment indicating re-adsorption processes, the determination of desorption isotherms was not performed.

The mass balance for the adsorption kinetics within 16 h ranged from 92.7 to 102.6% except for one soil (Refesol 03-G) with 84.6%. However, recovery of radioactivity within the adsorption isotherm experiment was 96.0 to 101.7% for all concentrations for soil Refesol 03-G.

Adsorption isotherms were calculated using the Freundlich equation. The calculated adsorption coefficients (K_F) in the adsorption tests ranged from 0.21 to 0.43 mL/g. Normalization to the organic carbon content of the soils resulted in K_{FOC} values from 9.0 to 20.2 mL/g (arithmetic mean 13.5 mL/g), indicating that MITC is weakly adsorbed to soil. The 1/n values obtained from the adsorption test ranged from 0.76 to 0.91 (arithmetic mean 0.830). The Freundlich adsorption isotherms had correlation coefficients (R^2) in the range of 0.947 to 0.998.

The sorption properties of [^{14}C]-MITC were studied in five soils using the batch equilibrium method ([redacted], 2009). The test soils included a range of textural classes, with pH values (in calcium chloride) of between 3.9 and 6.9 and organic carbon content between 0.6 and 3.9%.

A preliminary experiment was conducted to determine appropriate test parameters. The direct method (analysis of both water and soil phases) with a soil:solution ratio of 1:1 and equilibrium period of 8 hours were selected.

Initial concentrations of 0.05-5.0 mg/L were used in the main test. Mass balance was reported in the study as a total recovery of applied ^{14}C (including non-extracted residues) and ranged from 94.0-104.5 %. No transformation products were identified. Non-extracted residue ranged from 2.8% to 32.3%, indicating significant degradation of the test compound.

Freundlich isotherms were generated, allowing K_{Foc} and 1/n values to be determined. $K_{ads\text{-}Foc}$ values ranged from 20.4-71.7 (geomean = 36.3 mL/g, n = 5) and 1/n ranged from 0.85-0.91 (arithmetic mean = 0.882).

DMTU

The adsorption/desorption behaviour of ^{14}C-DMTU on soil was determined on five soils in the dark in the laboratory at 20 ± 2°C using the batch equilibrium method according to OECD Guideline No. 106 ([redacted], 2009).

In preliminary and screening tests the adsorption of the test item on the surfaces of the test vessels, the stability of the test item during the test period, the optimal soil-to-solution ratio and the appropriate equilibration times for adsorption/desorption were determined. Additionally, adsorption/desorption kinetics at a single concentration were determined.

Degradation of the test item was observed in all soils during the adsorption phase. Therefore, the radioactivity measured in the supernatant solutions was corrected by the amount of DMTU as analysed by HPLC/radiodetection. The amount adsorbed to the soils was considered completely as DMTU, since almost no radioactivity could be extracted from the soils and analysed. Once adsorbed to the soil the test item was strongly bound.

A non-GLP test showed that sterilization of soil did not slow down the rate of degradation. Therefore, for the advanced test a short adsorption and desorption time (2 hours each) was selected, but the soils were not sterilised.

The mass balance was established within the preliminary/screenings tests. After the adsorption step all radioactivity was recovered from the soil samples, with recoveries ranging from 94 to 99% for all soils. After the desorption step, again all of the radioactivity was recovered from the soil samples, with recoveries ranging from 95 to 97% for all soils.

The adsorption phase of the adsorption/desorption isotherm test was carried out using air-dried soils (soils II to V only) equilibrated in aqueous 0.01 M $CaCl_2$ solutions with a soil-to-solution ratio of 1:1 (1:1.5 for soil IV). DMTU was applied at five different initial test item concentrations (0.518, 0.131, 0.052, 0.013 and 0.005 mg/L) covering two orders of magnitude. Desorption was performed by supplying pre-adsorbed samples with fresh aqueous 0.01 M $CaCl_2$ solution. Adsorption and desorption took place for 2 hours equilibration time each.

Adsorption and desorption isotherms were calculated using the Freundlich equation. The calculated adsorption coefficients K_F ranged from 0.100 to 0.290 mL/g and the adsorption coefficients K_{FOC} (normalised to organic carbon content) ranged from 7 to 10 mL/g (geomean = 8 mL/g, n = 4). The coefficients of determination (r^2) for the linear regression of the Freundlich equation were not lower than 0.991. The 1/n values of 0.73-0.82 (arithmetic mean = 0.76 mL/g, n = 4) showed non-linearity for the adsorption process.

The geomean value for the desorption Freundlich coefficient $K_{des,FOC}$ was 22 mL/g. The higher Freundlich

isotherm coefficients for desorption indicate a partially irreversible sorption process.

The sorption properties of [^{14}C]-DMTU were studied in five soils using the batch equilibrium method ([redacted], 2011). The test soils included a range of textural classes, with pH values (in calcium chloride) of between 3.9 and 6.9 and organic carbon content between 0.6 and 3.9%.

A preliminary experiment was initially performed to determine the adsorption and desorption equilibration times for the main isotherm experiment. As a result of this experiment, equilibration times of 4 hours were selected. DMTU was added to 0.01 M calcium chloride solution (10mL total aqueous volume) pre-equilibrated with samples (10g dry weight each) of the five soil types. Five nominal concentrations of DMTU were used: 0.05, 0.15, 0.5, 1.5 and 5.0 mg/L. The samples were shaken in the dark at 20 ± 2°C.

Mass balance was reported in the study as a total recovery of applied ^{14}C (including non-extracted residues) and ranged from 90.8-95.9 %.

No transformation products were identified. Non-extracted residue ranged from 6.9% to 25.6% after adsorption and significant degradation was observed in HLPC analysis of the aqueous and soil extracts. The direct method was used in order to distinguish between adsorbed DMTU and any degradation products.

Freundlich isotherms were generated, allowing K_{Foc} and 1/n values to be determined. $K_{ads\text{-}Foc}$ values ranged from 5.23-22.5 (geomean = 7.97 mL/g, n = 5) and 1/n ranged from 0.70-0.94 (arithmetic mean = 0.834).

OECD 106 quality checks were conducted by the applicant. The values in the study were determined to be acceptable for all soils.

Soil photolysis

It is considered that metam and MITC will not be exposed to sunlight on the soil surface due to application by drip irrigation or incorporation in the soil and the short residence time of residues in soil. A soil photolysis study was not considered required during the previous renewal process.

Furthermore, for the renewal of metam sodium the example uses require soil to be covered by TIF sheeting for a period of at least 21 days. This prevents sunlight from reaching soil and therefore any soil residues are not subject to photolysis.

Mobility in soil

The sorption behaviour of MITC and DMTU were determined in the batch equilibrium studies described in Volume 3 B.8.1.2.1.2. Column leaching studies were not submitted by the Applicants.

Field studies

Soil dissipation studies are not triggered according to the data requirements due to the short residence time of residues in soil (DT_{50} lab <60 days and DT_{90} lab <200 days at 20°C for metam, MITC and DMTU). However, field soil dissipation studies were performed to investigate the degradation behaviour of MITC under TIF following application of metam ([redacted], 2020a, b, c).

The dissipation behaviour of residues of methyl isothiocyanate (MITC), after a single application of Metam® 510 to bare soil under protected conditions, was investigated at two sites, one in Southern Europe (Spain) and one in Central Europe (Hungary) ([redacted], 2020a). The sites were located in typical agricultural areas for application of soil disinfectants such as Metam® 510. The trial sites consisted of an untreated plot and a treated plot, the latter being subdivided into three subplots that were assigned for replicates.

The trial areas were irrigated before application with the aim to bring soil moisture content between 50% and 75% of the maximum water holding capacity according to the study plan. After completion of the experimental phase, it was found that soil moisture content should be between 50 % and 75% of field capacity (pF 2) at application for maximum fumigant efficacy which resulted in soil moisture exceeding field capacity, which is worst case in terms of degradation given that soil pore water promotes retention of the very soluble MITC and suppresses volatile loss. The product Metam® 510, containing the active ingredient metam sodium (generating MITC), formulated as a soluble concentrate (SL), was applied to the soil of the treated subplots at a nominal use rate of 300 L/ha corresponding to 153 kg a.i./ha by drip irrigation.

The application at the Spanish trial site was done on 13 June 2019 and at the Hungarian trial site on 14 June 2019, using at each site small plot drip irrigation equipment. The untreated plot and treated area were covered with a totally impermeable film (TIF) before application to retain the fumigant gases in the soil. The actual rates applied on the individual replicates ranged from 150.1 to 153.1 kg a.i./ha at the Spanish site with an average of 151.2 kg a.i./ha. On the Hungarian site the application rates ranged from 150.3 to 172.9 kg a.i./ha with an average of 158.5 kg a.i./ha. The TIF was removed from each trial area 42 days after application.

Soil specimens for residue analysis were taken at different intervals (at the Spanish site: 1 day before application (DBA), 0, 1, 3, 5, 13, 21, 42 days after application (DAA), 1, 3, 7 and 14 days after removal of TIF (DAR); at Hungarian site: 0 DBA, 0, 1, 3, 5, 14, 21, 42 DAA, 1, 3, 7 and 14 DAR). Soil cores of 10 cm depth increments were taken stepwise down to a maximum depth of 90 cm. Residue levels of MITC were monitored down to a depth of 90 cm starting from 0 DAA until 14 DAR (equivalent to 56 DAA).

Residues of MITC were predominantly found in the upper 40 cm soil layers during the study period accounting for 82.8 to 100.0 % (trial 560-001, Spain) and 99.7 to 100.0 % (trial 560-003, Hungary) of the total amounts found in the soil profile.

Some downward gas diffusion of MITC to deeper soil layers was observed.

MITC degraded rapidly under protected conditions. The mean residue within the top soil layer (0-40 cm) decreased at the Spanish trial site from 46.58 mg/kg at 0 DAA, 15.81 mg/kg at 1 DAA to 1.25 mg/kg at 5 DAA. In the same horizon at the Hungarian trial site, mean residues of 25.14 mg/kg at 0 DAA samples were detected followed by 10.21 mg/kg at 1 DAA sampling and 0.07 mg/kg at 5 DAA sampling event.

The dissipation behaviour of residues of methyl isothiocyanate (MITC), after a single application of Metam® 510 to bare soil under protected conditions, was investigated at one site in Southern Europe (Italy) ([redacted], 2020b). The site was located in typical agricultural areas for application of soil disinfectants such as Metam® 510. The trial site consisted of an untreated plot and a treated plot, the latter being subdivided into three subplots that were assigned for replicates.

The trial area was irrigated a few days before application with the aim to bring soil moisture content between 50 % and 75% of the maximum water holding capacity according to study plan. After completion of the experimental phase, it was found that soil moisture content should be between 50 % and 75% of field capacity (pF 2) at application for maximum fumigant efficacy which resulted in soil moisture exceeding field capacity, which is worst case in terms of degradation given that soil pore water promotes retention of the very soluble MITC and suppresses volatile loss. The product Metam® 510, containing the active ingredient metam sodium (generating MITC), formulated as a soluble concentrate (SL), was applied to the soil of the treated subplots at a nominal use rate of 300 L/ha corresponding to 153 kg a.i./ha by drip irrigation.

The application was done on 16 Oct 2019 using a small plot drip irrigation equipment. The untreated plot and treated area were covered with a totally impermeable film (TIF) before application to retain the fumigant gases in the soil. The actual rates applied on the individual replicates ranged from 152.1 to 152.9 kg a.i./ha with an average of 152.6 kg a.i./ha. The TIF was removed from the trial area 42 days after application.

Soil specimens for residue analysis were taken from 1 day before application (DBA), 0, 1, 3, 5, 14, 21, 42 days after application (DAA), 1, 3, 7 and 14 days after removal of TIF (DAR). Soil cores of 10 cm depth increments were taken stepwise down to a maximum depth of 90 cm. Residue levels of MITC were monitored down to a depth of 90 cm starting from 1 DBA until 14 DAR (equivalent to 56 DAA).

Residues of MITC were predominantly found in the upper 40 cm soil layer during the study period accounting for 95.3 – 100.0 % of the total amounts found in the soil profile.

Some downward gas diffusion of MITC to deeper soil layers was observed.

MITC degraded rapidly under protected conditions. The mean residue within the top soil layer (0-40 cm) decreased from mean residues of 21.3 mg/kg at 0 DAA followed by 18.07 mg/kg at 1 DAA, 2.98 mg/kg at 5 DAA and <0.1 mg/kg at 14 DAA.

The dissipation behaviour of residues of methyl isothiocyanate (MITC), after a single application of Metam 510 to bare soil under protected conditions, was investigated at one site in Northern Europe (Germany) ([redacted], 2020c). The site was located in typical agricultural areas for application of soil disinfectants such as Metam 510. The trial site consisted of an untreated plot and a treated plot, the latter being subdivided into three subplots that were assigned for replicates.

The trial area was irrigated a few days before application with the aim to bring soil moisture content to between 50% and 75% of the water retention characteristics at pF 2.0 according to study plan. The product Metam 510, containing the active ingredient metam sodium (generating MITC), formulated as a soluble concentrate (SL), was applied to the soil of the treated subplots at a nominal use rate of 300 L/ha corresponding to 153 kg a.i./ha by drip irrigation.

The application was done on 22 Apr 2020 using a small plot drip irrigation equipment. The untreated plot and treated area were covered with a totally impermeable film (TIF) before application to retain the fumigant gases in the soil. The actual rates applied on the individual replicates ranged from 149.5 to 155.0 kg a.i./ha with an average of 152.2 kg a.i./ha. The TIF was removed from the trial area 42 days after application.

Soil specimens for residue analysis were taken from 30 days before application (DBT), 0, 1, 3, 5, 13, 21, 42 days

after application (DAA), 1, 3, 6 and 13 days after removal of TIF (DAR). Soil cores of 10 cm depth increments were taken stepwise down to a maximum depth of 90 cm.

Residue levels of MITC were monitored down to a depth of 90 cm starting from 30 DBA until 13 DAR (equivalent to 55 DAA).

Residues of MITC were predominantly found in the upper 40 cm soil layer during the study period accounting for 99.7 – 99.9 % of the total amounts found in the soil profile.

Some downward gas diffusion of MITC to deeper soil layers was observed.

MITC degraded rapidly under protected conditions. The mean residue within the top soil layer (0-40 cm) decreased from mean residues of 36.3 mg/kg at 0 DAA followed by 13.6 mg/kg at 1 DAA, 5.7 mg/kg at 5 DAA and 1.2 mg/kg at 13 DAA.

The kinetic analysis of the new field soil dissipation data of MITC after application of metam by drip irrigation under protected conditions in Spain, Italy and Hungary ([redacted], 2020a, b) was summarized in [redacted] (2020a).

A summary of persistence/modelling endpoints for the field dissipation of MITC calculated before and after time-step normalisation to the reference temperature and moisture conditions are presented in the tables below.

Table 2.8.1-1: Summary of calculated non-normalised persistence endpoints for MITC

Field Dissipation Study	USDA Textural Class	Persistence DT_{50} (days)	Persistence DT_{90} (days)	Chi² (χ^2) %	Kinetic Model
Utrera, Spain[a]	Sand	0.761	2.53	10.1	SFO
Farmos, Hungary[a]	Sandy Loam	0.730	2.43	5.28	SFO
Dugliolo di Budrio, Italy[b]	Loam	2.03	6.73	9.68	SFO

[a] [redacted] (2020a).
[b] [redacted] (2020b).

Table 2.8.1-2: Summary of calculated modelling endpoints for MITC normalised to reference moisture and temperature conditions

Field Dissipation Study	USDA Textural Class	Modelling DT_{50} (days) (20°C; pF2)	Modelling DT_{90} (days) (20°C; pF2)	Chi² (χ^2) %	Kinetic Model
Utrera, Spain[a]	Sand	1.52	5.04	9.40	SFO
Farmos, Hungary[a]	Sandy Loam	0.417	1.38	8.99	SFO
Dugliolo di Budrio, Italy[b]	Loam	2.99	9.93	8.77	SFO

[a] [redacted] (2020a).
[b] [redacted] (2020b).

The kinetic analysis of the new field soil dissipation data of MITC after application of metam by drip irrigation under protected conditions in Germany ([redacted], 2020c) is summarized in [redacted] (2020b).

A summary of persistence/modelling endpoints for the field dissipation of MITC calculated before and after time-step normalisation to the reference temperature and moisture conditions are presented in the tables below.

Table 2.8.1-3: Summary of calculated non-normalised persistence endpoints for MITC

Field Dissipation Study	USDA Textural Class	Persistence DT_{50} (days)	Persistence DT_{90} (days)	Chi² (χ^2) %	Kinetic Model
Kalkar, Germany[a]	Sandy Loam	0.954	3.17	20.7	SFO

[a] [redacted] (2020c).

Table 2.8.1-4: Summary of calculated modelling endpoints for MITC normalised to reference moisture and temperature conditions

Field Dissipation Study	USDA Textural Class	Modelling DT_{50} (days) (20°C; pF2)	Modelling DT_{90} (days) (20°C; pF2)	Chi² (χ^2) %	Kinetic Model
Kalkar, Germany[a]	Sandy Loam	0.761	2.53	22.2	SFO

[a] [redacted] (2020c).

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Degradation in water/sediment

Metam

The aerobic aquatic metabolism of [^{14}C]-metam-potassium was studied in non-sterile water/sediment mixtures ([redacted], 1993). A determination of the microbial activity demonstrated aerobic viability in the test systems. Test systems, maintained under aerobic conditions, were treated with [^{14}C]-metam-potassium at approximately 26 ppm and incubated in the dark at 25.0 ± 1 °C. Aerobic conditions were verified by measuring the dissolved oxygen content of the test systems. Volatile metabolites formed during the incubation period were flushed with air into traps containing activated charcoal. The overall average material balance in the study ranged from 91.2 to 103.7%. Residues in the aqueous phase were 50.5 - 55.8% AR from 4.5 to 122 minutes then decreased to 8.5% AR at 480 minutes. Extractable residues in the sediment were 16.2 – 22.7% AR from 4.5 to 122 minutes then decreased to 4.8% AR at 480 minutes. Non-extractable residues were 24.0 – 33.0% AR from 4.5 to 122 minutes then decreased to 14.4% AR at 480 minutes.
Volatiles were on average of 43.1% AR at 240 minutes and 63.6% AR at 480 minutes. At each of the sampling intervals in which volatiles were trapped in charcoal, 100% of the charcoal extractable radioactivity was found to be MITC by HPLC/radiodetection analysis. No other radiolabelled volatiles were observed. No CO_2 determination was performed (no NaOH trap present).
The major metabolite observed was Methylisothiocyanate (MITC), which represented 74.4% of the initial radioactivity at the 480 minute sampling point. A transient metabolite, identified as 1,3-Dimethylthiuramdisulfide (DMTD), reached a maximum of 29.0% of the initial radioactivity at 122 minutes, then declined to 13.6% at 240 minutes and was not detected at 480 minutes. Several other minor metabolites that were formed were identified as 1,3-Dimethylurea (DMU), 1,3-Dimethylthiourea (DMTU) and methylcarbamo(dithioperoxo)thioate (MCDT). None of these metabolites exceeded 4.2% AR at any sampling interval.

Metam-potassium metabolised with an estimated half-life of 19.72 minutes (k = -0.03514 min^{-1}) under aerobic aquatic conditions.

MITC

[^{14}C]-MITC (radiochemical purity >95%) was added to the water layer of two water/sediment systems at a nominal concentration of 58 mg/L ([redacted], 2013). Tests were conducted using static and aerated conditions on each of the two water/sediment systems. Aquatic sediment systems were acclimatised under aerobic conditions prior to MITC application until reasonable stability had been established with respect to the pH, oxygen concentration and redox potential in the water and the pH and redox potential in the sediment. Following application samples were incubated in the dark at 12 °C, under both static and aerated conditions for periods of up to 31 days and 15 days for static and aerated systems, respectively. Due to the high volatility of MITC, this lower temperature was considered more appropriate than the higher temperature of 20 °C that is typically used in studies on plant protection products. The study was conducted at a temperature of 12 °C. This followed a recommendation in the 2011 EFSA conclusion to perform studies at lower temperatures to provide a representative worst-case estimate for the effect of volatilisation. Sediment and water phases were analysed separately. Radiolabelled volatile components were trapped and quantified (aerated system only) and a material balance obtained for each sample.
Under static conditions MITC dissipated slowly from the water of aquatic sediment systems with $DissT_{50}$ values of 32.2 days (Calwich Abbey Lake) and 68 days (Swiss Lake). Decline in the overall system corresponded to $DegT_{50}$ values of 83.5 and 168 days, respectively.
Under aerated conditions MITC dissipated quickly from the water of aquatic sediment systems by volatilisation and partitioning to sediment with $DissT_{50}$ values of 5.96 days (Calwich Abbey Lake) and 3.0 days (Swiss Lake). Decline in the water/sediment system corresponded to $DissT_{50}$ values of 15.0 and 3.65 days, with most MITC dissipating via volatilisation.
MITC degraded slowly resulting in the formation of three unidentified minor components (<1.5%) and incorporation into bound sediment fractions. Degradation to CO_2 was minimal (max. 0.4% AR).

Hydrolysis

Metam

The rate of hydrolysis of [^{14}C]-metam-sodium has been determined in aqueous solutions buffered at pH 5, 7, and 9 ([redacted], 1990). The studies were performed in the dark under sterile conditions at concentrations ranging from 57 to 65 mg/L. Dilute [^{14}C]-metam-sodium degrades rapidly in water at 25 °C. The half-lives in this concentration range were 2, 2, and 4.5 days at pH 5, 7, and 9, respectively. The major degradate formed at pH 5 was methyl isothiocyanate (MITC; 18% of the initial radioactivity). The major degradate formed at pH 7 was MITC (60% of the initial radioactivity). At pH 9, two major degradates formed, with MITC accounting for 20% and sodium methylcarbamo(dithioperoxo)thioate (MCDT) accounting for 16% of the initial radioactivity.

MITC

[^{14}C]-MITC was shown to degrade significantly at every pH and temperature with the exception of pH 4 and 7 at 15 °C, precluding the calculation of half-life data for these test groups ([redacted] 2005). DT_{50} and DT_{90} values could not be determined for samples incubated at pH 9 and 50 °C as the kinetics were not linear. DT_{50} and DT_{90} values were established for pH 4 (25 °C and 50 °C), pH 7 (25 °C and 50 °C) and pH 9 (15 °C and 25 °C). DT_{50} values for MITC were 44, 50 and 11 days at pH 4, 7 and 9 and 25 °C, respectively.
Degradation was more extensive at higher temperatures and higher pH concomitant with higher hydroxyl ion concentration.

All degradants which were present at greater than 10% of the applied radioactivity in samples at pH 7 and 9 were characterised by LC-MS. Samples incubated at pH 4 and 50 °C also showed significant degradation but it was not possible to analyse these samples by MS because of the rapid degradation. However, the degradants observed were chromatographically consistent with those observed at pH 7 and 9 and thus it is considered that all significant degradants of [^{14}C]-MITC have been characterised. The following products were found to exceed 10% of applied radioactivity (Unknown A, Unknown B, Unresolved Material, Unknown C, Unknown D, DMTU, Unknown 4, DMU, MDTCA and Unknown F).

Photochemical degradation

Aqueous photolysis

Metam

The photolysis of [^{14}C]-metam-sodium (sodium methyldithiocarbamate) at 25 °C has been examined in an aqueous solution buffered at pH 7 (Spurgeon, 1990). A xenon arc lamp was used to irradiate samples for up to 24.6 min. Additional samples were incubated in the dark. The test was performed under sterile conditions with concentrations of [^{14}C]-metam-sodium at 52 mg/L (main test) and 100 mg/L (additional test). The half-life was 11.9 min which is equivalent to 27.8 min of natural summer sunlight at Richmond, CA (latitude 37° 56' N). The average recovery, based on radiochemical analysis, was 94%. A combination of chromatographic and spectroscopic methods was used to identify the photoproducts of [^{14}C]-metam-sodium. The photoproducts formed were syn and anti-forms of N-methylthioformamide (up to 22.3%), methylamine (up to 17.5%), methylisothiocyanate (MITC, up to 16.0%), and sodium methylcarbamo (dithioperoxo)thioate (MCDT, up to 14.1%).

MITC

The photolysis of [^{14}C]-MITC was investigated in sterile pH 4 potassium biphthalate buffer ([redacted], 2019). [^{14}C]-MITC was applied, at a concentration of 0.001 mg/mL, to the buffer solution in individual photolysis vessels. The treated solutions were irradiated using light from a xenon arc lamp, which emitted light that was filtered to give a spectral distribution close to that of natural sunlight at a mean intensity of 49.6 W/m^2 (300-400 nm). The samples were maintained at 25 ± 2°C and were continuously irradiated for periods up to 5 days. A control sample was incubated concurrently under similar conditions in the dark.

Irradiated samples were taken for analysis at 3 intervals during irradiation. A dark control sample was taken for analysis at termination alongside the final irradiated sample.

The mean mass balance from the irradiated samples was 97.3% applied radioactivity (range 95.7-98.4%) and the mass balance from the dark control sample was 93.6%. No single transformation product was observed at >3.8% of the applied dose.

The DT_{50} was calculated from data generated from the irradiated samples using a single first order model and was

determined to be approximately 39 d ($k_{irradiated}$ = 0.01771). Subtracting degradation due to hydrolysis in the dark (reported in a separate study), the approximate laboratory direct photolysis rate constant was calculated as 0.00221, which results in an estimated half-life for degradation by photolysis of 329 d. The negligible degradation under irradiated aqueous conditions demonstrates that photolysis is an insignificant degradation route for MITC.

Ultimately the results obtained from the preliminary range finding study demonstrated that [^{14}C]-MITC:

- May be considered stable under photolytic conditions.
- Photolysis is not a significant degradation pathway for MITC
- The minor degradation observed during the test may be attributed to hydrolysis.
- Degradation products observed were present in both irradiated and dark control samples at similar levels – demonstrating no photolysis specific transformation products

Conclusion on rapid degradation in the aquatic environment

According to the Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (ECHA, 2017), preferred data types for the decision on rapid degradability are screening 28-day ready biodegradation studies and surface water simulation tests. When these preferred data types are not available rapid degradation may be demonstrated by soil or aquatic sediment simulation tests.

Metam

Based upon an OECD 301 D study ([redacted], 2008), metam is considered to be **not readily biodegradable**. Rapid transformation was measured in available simulation studies in soil (metam-sodium) as well as water/sediment (metam-potassium) with DT_{50} values of 0.0059 - 0.0324 d and 0.0456 d, respectively. The low DT_{50} values are below the trigger value for rapid degradation. However, they do not account for mineralisation but for transformation to the major metabolite MITC. Thus, the conclusion on rapid degradation of metam should be derived from the available data for the metabolite MITC. Based on the available data on aerobic transformation/degradation in water-sediment systems, MITC is not considered readily biodegradable.

MITC

Based on the available data on aerobic transformation/degradation in water-sediment systems rapid biodegradation of MITC is not expected. Within the whole system the DT_{50}-values were clearly above the limit of 16 d (83.5 d - 1680 d at 12 °C). In the aerated system the DT_{50}-values dropped below 16 d (3.65 – 15.0 d at 12 °C) reflecting dissipation. Degradation to CO_2 was minimal (max. 0.4% AR) in this study.
Thus, in conclusion metam is considered to transform rapidly to the major metabolite MITC in the aquatic environment. MITC degradation is slow in aquatic water/sediment systems with little evidence of mineralisation in the system. Based on the available data on aerobic transformation/degradation in water-sediment systems, MITC is **not considered readily biodegradable**.

2.8.2.1 Rapid degradability of organic substances

Table 67: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study[1]	Remarks	Reference
Metam				
Ready biodegradability				
OECD 301 D Commission Regulation (EC) No 440/2008, Method C.4-E of May 30, 2008: Closed Bottle Test (EEC Publication N° L	Under the test conditions the mean percentage biodegradation of Metam Sodium 510 g/L reached a maximum of 9 % (based on $ThOD_{NH4}$) and 7 % (based on $ThOD_{NO3}$) during 28 days of incubation.	Acceptable	Test item: Metam Na 510 SL; 41.7% (w/w) [metam-sodium] Activated sludge (microorganisms from a domestic waste water treatment plant) supplied by the sewage	[redacted] (2018), Report No. 134711161

Method	Results*	Key or Supportive study[1]	Remarks	Reference
142/497-502, May 2008) GLP	The percentage biodegradation did not reach 60 % within the 10-day window or after 28 days of incubation. The test item can therefore be considered to be not readily biodegradable.		treatment plant Bensheim, Germany.	
Aerobic Transformation/Degradation in Soil				
US EPA Pesticide Assessment Guidelines Subdivision N, Paragraph 162-1, 1982 SETAC Document "Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides (March 1995)"	DT_{50}-values for different soil types at test temperature: Sandy Loam 2 S402: 17 min (20 °C), 36.14 min (12 °C**) Sandy Loam 1 S403: 4 min (20 °C), 8.50 min (12 °C**) Silt Loam S405: 11 min (20 °C), 23.33 min (12 °C**) Clay Loam S404: 9 min (20 °C), 19.15 min (12 °C**) Mean (All Soils, 20 ºC): 10 min (20 °C), 21.17 min (12 °C**) Sandy Loam 2 S402: 17 - 22 min (10 °C), 36.14 - 64.66 min (12 °C**)	Acceptable	Test item: ^{14}C Metam-sodium DT_{50}-values were re-calculated to 12 °C using Arrhenius equation.	[redacted] (2004), Report No. 24253
Aerobic Transformation/Degradation in Water/Sediment				
EPA Pesticide Assessment Guidelines 40 CFR 158, Subdivision N, Section 162-4	DT_{50}: 19.72 min (0.33 h) at 25.0 ± 1 °C; 0.0456 d (12 °C**) MITC and 1,3-Dimethylthiuramdisulfide (DMTD) are formed (major metabolites).	Supportive information	Test item: ^{14}C-PNMDC (potassium N-methyldithiocarbamate) Texture of sediment: sandy loam DT_{50}-value was re-calculated to 12 °C using Arrhenius equation.	[redacted] (1993), Report No. ME 9100132
Hydrolysis				
US-EPA Subdivision N Series 161-1.Publication 540/9-82-021, October 18, 1982) GLP	DT_{50} pH 5, 7: 2 d (25 ± 1 °C); 6.66 d (12 °C**) DT_{50} pH9: 4.5 d (25 ± 1 °C); 15 d (12 °C**)	Acceptable	Test material: ^{14}C Metam-sodium DT_{50}-values were re-calculated to 12 °C using Arrhenius equation.	[redacted] (1990), Report No. RR 90-110B

Method	Results*	Key or Supportive study[1]	Remarks	Reference
	MITC is formed at all pH values tested (major metabolite). MCDT is formed at pH 9 (major metabolite)			
Photochemical degradation				
Subdivision N, US EPA, Guideline Series 161-2 (October 1982) GLP	DT_{50}: 11.9 min, equivalent to 27.8 min of natural summer sunlight at Richmond, CA (latitude 37° 56' N) at 25 °C The photoproducts formed were syn and anti-forms of N-methylthioformamide (up to 22.3%), methylamine (up to 17.5%), methylisothiocyanate (MITC, up to 16.0%), and sodium methylcarbamo (dithioperoxo)thioate (MCDT, up to 14.1%).	Partially acceptable (kinetic assessment does not follow the FOCUS guidance)	Test item: ^{14}C Metam-sodium	[redacted] (1990), Report No. RR-90-091B
OECD Guideline 316: Phototransformation of chemicals in water (October 2008)	DT_{50}: 20.9 hours at 25°C under sterile conditions, using SFO kinetics. Quantum yield of metam-sodium: 0.00625 using a PNA/pyridine actinometer Component A (up to 16.74%) had similar chromatographic properties to 2-imidazolethianon, although this was not confirmed. Component B (max. 39.28%) was a multicomponent mixture, co-eluting with MTU and DMTU in HPLC. Only DMTU was seen in TLC. MITC co-eluted with metam-sodium (component C)	Supportive	The HPLC method could not distinguish between metam and MITC, DT_{50} and quantum yield are thus not for metam only. DMTU and MTU co-eluted with degradation products observed in HPLC. DMTU was also identified in TLC, while DMU was not.	[redacted] (2013), Report No. QBR102477/1
MITC				
Aerobic Transformation/Degradation in Soil				
US EPA Pesticide Assessment Guidelines Subdivision N,	**DT_{50}-values for different soil types:**	Acceptable	Test item: ^{14}C Metam-sodium	[redacted] (2004),

Method	Results*	Key or Supportive study[1]	Remarks	Reference
Paragraph 162-1, 1982 SETAC Document "Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides (March 1995)" GLP	Sandy Loam 2 S480: 2.78 d (20 ºC); 5.90 d (12 °C**) Sandy Loam 1 S500: 2.94 d (20 ºC); 6.24 d (12 °C**) Silt Loam S490: 0.97 d (20 °C); 2.06 d (12 °C**) Clay Loam S479: 1.91 d (20 ºC); 4.05 d (12 °C**) Mean (All Soils): 2.15 d (20 ºC); 4.56 d (12 °C**) Sandy Loam 2 S480: 8.31 (10°C)		MITC is a major soil metabolite of metam-sodium. DT_{50}-values were re-calculated to 12 °C using Arrhenius equation.	Report No. 24253
OECD Guideline 307 (2002), US EPA OPPTS 835.4100 (2008) GLP	**DT_{50}-values for different soil types:** Clay Loam/Silt Loam/Loam S777: 1.2 - 1.6 d (20 °C), 2.5 - 3.4 d (12 °C) Sandy Loam 1 S778: 5.0 - 7.6 d (20 °C), 10.6 - 16.1 d (12 °C) Sandy Loam 2 S779: 4.7 - 5.6 d (20 °C), 10.0 - 11.9 d (12 °C) Loam/ Clay Loam S780: 4.8 - 4.9 d (20 °C), 10.2 - 10.4 d (12 °C)	Acceptable	Test item: [^{14}C-Thiocarbonyl]-MITC DT_{50}-values were re-calculated to 12 °C using Arrhenius equation.	[redacted] (2014), Report No. 34284
Aerobic Transformation/Degradation in Water/Sediment				
OECD 308 GLP	**$DegT_{50}$ (whole system, 12 °C)** Swiss Lake (static): 168 d Calwich Abbey Lake (static): 83.5 d Swiss Lake (aerated): 3.65 d Calwich Abbey Lake (aerated): 15.0 d **$DissT_{50}$ (water, 12 °C)** Swiss Lake (static): 68 d Calwich Abbey Lake (static): 32.2 d	Acceptable	Test item: ^{14}C MITC DT_{50}-values were re-calculated to 12 °C using Arrhenius equation.	[redacted] (2013), Report No. PQB0017

Method	Results*	Key or Supportive study[1]	Remarks	Reference
	Swiss Lake (aerated): 3.0 d Calwich Abbey Lake (aerated): 5.96 d			
Hydrolysis				
OECD 111 (October 2002) GLP	DT_{50} pH4: 44 d (25 ± 0.5 °C); 146.5 d (12 °C**) DT_{50} pH7: 50 d (25 ± 0.5 °C); 166.5 d (12 °C**) DT_{50} pH9: 11 d (25 ± 0.5 °C); 36.6 d (12 °C**)	Acceptable, except for the calculation of degradation rates (to be updated to follow the FOCUS guidance)	Recoveries at Day 30 were <90% AR at 25 °C and 50 °C for pH 4 and at 50 °C for pH 7 but study considered acceptable.	[redacted] (2005), Report No. 24450
OECD 111 GLP	DT_{50} pH4: 322 d (20 ± 1 °C); 684 d (12 °C**) DT_{50} pH7: 165 d (20 ± 1 °C); 350 d (12 °C**) DT_{50} pH9: 16.1 d (20 ± 1 °C); 34.2 d (12 °C**)	Acceptable	Recovery at pH 4 was below acceptable levels due to volatile losses. As quantitative recoveries were achieved at pH 7 and 9, the low values in the pH 4 experiment were attributed to the loss of a volatile hydrolysis product rather than to loss of methyl isothiocyanate. As such, the recoveries were regarded as quantitative and this does not affect the validity of the study.	[redacted] 2009, Report No. LNO0005
Aqueous photolysis				
OECD 316 (October 2008) GLP	DT_{50} at pH4: 39 d (irradiated samples, 25 ± 2 °C) DT_{50} at pH4: 329 d (direct photolysis, 25 ± 2 °C)	Acceptable	Test item: ^{14}C MITC No single transformation product was observed at >3.8% of the applied dose.	[redacted] (2019), Report No. 815511

** data on full mineralization should be reported*
*** Test temperature was corrected using Arrhenius equation.*

2.8.2.1.1 Ready biodegradability

Metam

The test item Metam Na 510 SL was investigated for its ready biodegradability in a Closed Bottle Test over a period of 28 days ([redacted], 2018). The biodegradation was followed by the oxygen uptake of the microorganisms during an exposure period of 28 days. A reference item was tested simultaneously under the same conditions as the test item, and functioned as a procedure control.
Under the test conditions the mean percentage biodegradation of Metam Na 510 SL reached a maximum of 9% (based on $ThOD_{NH4}$) and 7 % (based on $ThOD_{NO3}$) during 28 days of incubation. The percentage biodegradation did not reach 60% within the 10-day window or after 28 days of incubation. The test item can therefore be considered to be **not readily biodegradable**.

MITC

No relevant data available.

2.8.2.1.2 BOD5/COD

No relevant data available.

2.8.2.2 Other convincing scientific evidence

2.8.2.2.1 Aquatic simulation tests

No relevant data available.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No relevant data available relevant for C&L.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

No relevant data available.

2.8.2.2.4 Soil and sediment degradation data

Please refer to 2.8.1.

2.8.2.2.5 Hydrolysis

Please refer to 2.8.2.

2.8.2.2.6 Photochemical degradation

Aqueous photolysis

Metam

The photolysis of [^{14}C]-metam-sodium (sodium methyldithiocarbamate) at 25 °C has been examined in an aqueous solution buffered at pH 7 (Spurgeon, 1990). A xenon arc lamp was used to irradiate samples for up to 24.6 min. Additional samples were incubated in the dark. The test was performed under sterile conditions with concentrations of [^{14}C]-metam-sodium at 52 mg/L (main test) and 100 mg/L (additional test). The half-life was 11.9 min which is equivalent to 27.8 min of natural summer sunlight at Richmond, CA (latitude 37° 56' N). The average recovery, based on radiochemical analysis, was 94%. A combination of chromatographic and spectroscopic methods was used to identify the photoproducts of [^{14}C]-metam-sodium. The photoproducts formed were syn and anti-forms of N-methylthioformamide (up to 22.3%), methylamine (up to 17.5%), methylisothiocyanate (MITC, up to 16.0%), and sodium methylcarbamo (dithioperoxo)thioate (MCDT, up to 14.1%).

MITC

The photolysis of [^{14}C]-MITC was investigated in sterile pH 4 potassium biphthalate buffer ([redacted], 2019). [^{14}C]-MITC was applied, at a concentration of 0.001 mg/mL, to the buffer solution in individual photolysis vessels. The treated solutions were irradiated using light from a xenon arc lamp, which emitted light that was filtered to give a spectral distribution close to that of natural sunlight at a mean intensity of 49.6 W/m^2 (300-400 nm). The samples were maintained at 25 ± 2°C and were continuously irradiated for periods up to 5 days. A control sample was incubated concurrently under similar conditions in the dark.

Irradiated samples were taken for analysis at 3 intervals during irradiation. A dark control sample was taken for analysis at termination alongside the final irradiated sample.

The mean mass balance from the irradiated samples was 97.3% applied radioactivity (range 95.7-98.4%) and the mass balance from the dark control sample was 93.6%. No single transformation product was observed at >3.8% of the applied dose.

The DT_{50} was calculated from data generated from the irradiated samples using a single first order model and was determined to be approximately 39 d ($k_{irradiated}$ = 0.01771). Subtracting degradation due to hydrolysis in the dark (reported in a separate study), the approximate laboratory direct photolysis rate constant was calculated as 0.00221, which results in an estimated half-life for degradation by photolysis of 329 d. The negligible degradation under irradiated aqueous conditions demonstrates that photolysis is an insignificant degradation route for MITC.
Ultimately the results obtained from the preliminary range finding study demonstrated that [^{14}C]-MITC:

- May be considered stable under photolytic conditions.
- Photolysis is not a significant degradation pathway for MITC
- The minor degradation observed during the test may be attributed to hydrolysis.
- Degradation products observed were present in both irradiated and dark control samples at similar levels – demonstrating no photolysis specific transformation products

2.8.2.2.7 Other / Weight of evidence

Not relevant.

2.8.3 Summary of fate and behaviour in air

Metam-sodium is expected to be very rapidly degraded in air ([redacted] 2001), with a DT_{50} of 1.997 hours (assuming a hydroxyl radical concentration of 1.5 x 10^6 OH radicals/cm^3).

MITC, its major metabolite, has a high vapour pressure and is released as a gas upon degradation of metam-sodium. Consequently, there is known to be volatile loss of MITC from soils treated with metam-sodium.

MITC has been shown to have a worst case DT_{50} in air of between 4.8 and 6.3 days ([redacted], 2010b). The estimation of removal from air by deposition performed on the basis of the physicochemical properties indicate that MITC is likely to be found in air, but with a high affinity for water. Therefore, it is likely to be partitioned predominately between the air and atmospheric water (including aerosols). This implies that MITC is susceptible to both wet and dry deposition and that it will be readily removed from the air column by precipitation.

Short degradation rates of MITC in soil (DT_{50} 2.1 days) and in water (hydrolytic DT_{50} at pH 7 and 25°C of 50 days; [redacted] 2005) as well as its low bioaccumulation potential (MITC's relatively low LogP: 1.05, [redacted], 1997) indicate that the risk related to residues re-deposited in remote environments is negligible. MITC does not contain halogen atoms and has not been considered by Montreal convention as a compound responsible of ozone layer depletion.

2.8.3.1 Hazardous to the ozone layer

Table 68: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
Tiered approach from FOCUS Air Working Group (FOCUS, 2008)	Short degradation rates of MITC in soil (DT_{50} 2.1 days) and in water (hydrolytic DT_{50} at pH 7 and 25°C of 50 days; [redacted], 2005) as well as its low bioaccumulation potential (MITC's relatively low LogP: 1.05, [redacted], 1997) indicate that the risk related to residues re-deposited in remote environments is negligible. MITC does not contain halogen atoms and has not been considered by Montreal convention as a compound	From DAR (2010)	[redacted], 2010b, Report No. CEA.536

Method	Results	Remarks	Reference
	responsible of ozone layer depletion.		
Method	**Results**	**Remarks**	**Reference**

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Due to its relatively low volatility Metam (incl. -potassium and -sodium) is highly unlikely to deplete the stratospheric ozone layer. MITC has a potential for volatilization based on its vapour pressure of 1739 Pa (20 °C, OECD 104). However, there is no evidence based on the chemical structure and substance properties that MITC may have a potential for depleting the ozone layer.

2.8.3.1.2 Comparison with the CLP criteria

A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.
The relatively low volatility of Metam (incl. -potassium and -sodium) precludes an ozone-layer-depleting potential. Even though MITC has a potential for volatilization it is not considered to have any impact on the ozone layer. Both substances are not listed in Annex I to Regulation (EC) No 1005/2009 having an Ozone Depleting Potential (ODP) greater or equal to the lowest ODP (i.e. 0.005) and are therefore **not subject to classification as Hazardous to the Ozone Layer** (Category 1).

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified - conclusive but not sufficient for classification.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Monitoring data from across the EU member states where metam is/was used and where it was considered likely that surface and groundwater monitoring data would be collected are limited, with data only currently available from two MS: France and The Netherlands.

For France, following analysis of the datasets from the ADES (groundwater) and NAÏADES (surface water) eaufrance websites, for the period 2000 – 2019, the following values were deemed to be valid:

- For the presence of metam-sodium in surface water, 4 results were considered to be >LOQ with values ranging from 2.4 to 3.2 µg/L. For groundwater, 846 analyses were reported as having analysis made and results validated at "Niveau 2", but all were <LOQ.
- For the presence of MITC in surface water, 1701 samples were recorded as "Niveau 2". All but 6 of these analyses were <LOQ or <LOD. In groundwater, MITC was included in 14865 analyses with a validation of "Niveau 2". Only 2 analyses had detectable MITC in the analytically valid range for those analyses with values of 0.25 and 1.06 µg/L (0.013% of samples analysed).

For The Netherlands, analysis of datasets from the 'Groundwater Atlas for Pesticides' and the 'Atlas of Pesticides in surface water', for the period 2000 – 2016 (groundwater) and 2000 – 2018 (surface water) yielded the following:

- Whilst groundwater monitoring data had been recorded for the active metabolite MITC, all analysed samples were <LOQ (4501 groundwater samples).
- For surface water, monitoring data had also been recorded for the active metabolite MITC, and all analysed samples were <LOQ (888 surface water samples).

2.8.5 Definition of the residues in the environment requiring further assessment

Definition of the residue for risk assessment

Compartment	Component
Soil*	MITC, DMTU (impurity)
Groundwater	MITC, DMTU (impurity)
Surface water	MITC

Sediment	MITC
Air	MITC

* Taminco submitted a rationale to demonstrate that DMTU is not pertinent in soil for use in permanent structures.

Remark: the definitions of the residue for risk assessment were not equivalent for Taminco and Lainco. This proposal is the opinion of the RMS and co-RMS based on the evaluation of both dossiers.

Definition of the residue for monitoring

Compartment	**Component**
Soil	MITC
Groundwater	MITC
Surface water	MITC
Sediment	MITC
Air	MITC

2.8.6 Summary of exposure calculations and product assessment

The active substance metam is available in two variants: metam-sodium (sodium methyl-dithiocarbamate) and metam-potassium (potassium methyldithiocarbamate). Based on the Tier II equivalence assessment, both variants are considered to be equivalent (please refer to Vol. 4 for further details). The variant metam-sodium is considered to be the reference source.

Metam is an MITC generator and this is the moiety that has the biological activity. Furthermore, negligible exposure is expected to metam. Consequently, MITC is considered as the relevant molecule for the risk assessments. Metam sodium undergoes rapid and complete degradation to MITC (>99% degradation within 2 hours of application), with 100 % molar conversion equivalent to 56.6% w/w conversion. Therefore, MITC is treated as an active substance in PEC_s calculations.

The GAP considered in the risk assessment of metam-sodium in this section are summarised in Table 2.8.6-1.

Table 2.8.6-1: Summary of use pattern

Product	**Crop treated (before planting)**	**F, Fn, Fpn G, Gn, Gpn or I****	**Max. application rate of product (L/ha)**	**Max. application rate of metam-sodium (kg/ha)**	**Max. application rate of MITC (kg/ha)[a]**	**Application Method**	**Application Frequency/timing**
Metam Na 510 SL (Vol. 3 CP_ Metam Na 510 SL_B-8)	Lettuce	G	300	153 (172*)	86.6	Drip irrigation with gas tight (totally impermeable) TIF film (6 weeks) in permanent structures.	1 application every 3 years
	Ornamentals	G	300	153 (172*)	86.6		
	Baby leaf	G	150-200	77-102 (86-115*)	43.6 – 57.7		
Metam Sodium 51% SL (Vol. 3 CP_ Metam Sodium 51% SL_B-8)	Pepper	G	600	306 (254*)	173.2	Drip irrigation in combination with the use of Total Impermeable Film (TIF) for at least 21 days in permanent structure and	1 application every 3 years Period: spring to winter. Pre-plant or pre-sowing.

						walk-in tunnels.	
	Potato	F	300	153 (172*)	86.6	Soil injection (15-20 cm depth) in combination with Total Impermeable Film (TIF) for at least 21 days.	1 application every 3 years Period: spring to winter. Pre-plant or pre-sowing.
	Carrot	F	300	153 (172*)	86.6		
	Onion	F	300	153 (172*)	86.6		

* Application rate expressed for the equivalent potassium salt product [a]

[a] Molar mass of MITC relative to metam sodium of 0.566. Molar mass of MITC relative to metam potassium of 0.503

** F: professional field use, Fn: non-professional field use, Fpn: professional and nonprofessional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

There are no formulants that could potentially influence long-term processes, such as degradation and distribution. Therefore, for the purposes of the risk assessments it is assumed that formulants do not influence the fate and behaviour of an active substance in the environment and are not considered further.

The kinetic endpoints recommended to be used in the exposure assessment are given below in the Table 2.8.6-2.

Table 2.8.6-2: Kinetic endpoints for MITC and the impurity DMTU recommended to be used in the exposure assessment.

Type of assessment	Compound	Recommended kinetic endpoints		
		Soil DT_{50} [days]	Type of value[1]	Kinetic model
Soil exposure assessment (PEC_{SOIL})	MITC	3.24 d (at 153 kg metam sodium/ha) 5 d (at 306 kg metam sodium/ha)	Worst-case of lab values ([redacted], 2010b; [redacted] 2014)	SFO
	DMTU	0.308	Worst-case lab value	DFOP
Groundwater exposure assessment (PEC_{GW})	MITC	2.1 d (at 153 kg metam sodium/ha) 3.2 d (at 306 kg metam sodium/ha)	Geomean of lab values ([redacted], 2010b; [redacted] 2014)	SFO
	DMTU	0.202	Geomean of lab values ([redacted], 2010a; [redacted] 2011; [redacted], 2011)	Pseudo-SFO
Surface water exposure assessment (PEC_{SW}/PEC_{SED})	MITC	2.1 d (at 153 kg metam sodium/ha) 3.2 d (at 306 kg metam sodium/ha)	Geomean of lab values ([redacted], 2010b; [redacted] 2014)	SFO
	DMTU	0.202	Geomean of lab values ([redacted], 2010a; [redacted] 2011; [redacted], 2011)	Pseudo-SFO

[1] DT_{50} values are normalized to 20°C and pF 2

Soil

There is no accumulation in soil expected for metam sodium, MITC and DMTU.

The decline of residues in soil was simulated using a Microsoft Excel spreadsheet. SFO decline kinetics were modelled. PEC_{SOIL} values immediately after application were calculated using current FOCUS guidance (FOCUS, 1997) with the following equation:

$$PEC_{initial}\ (mg/kg) = \frac{A \times (1 - F)}{100 \times d \times \rho}$$

Where:

A = Application rate (g/ha)

F = Fraction intercepted by crop (-)
d = Depth of field soil layer (5 cm)
ρ = Dry bulk density (1.5 g/cm^3)

PEC_{SOIL} values at specific times (t) after application were calculated as:

$$PEC_t = PEC_{initial}\, e^{-kt}$$

Where *k* is the first-order rate constant
Time weighted average (TWA) PEC_{SOIL} values were calculated using a "moving-window" approach. The average PEC_{soil} within a day was calculated as follows:

$$\text{Average PEC over a day (mg/kg)} = \frac{\text{Actual PEC at start of day} \times (1-e^{-k})}{k}$$

TWA PEC_{SOIL} over the moving window were then calculated as the simple numerical average of these daily values.

Field and walk-in tunnels
It concerned the product Metam Sodium 51% SL (see Vol.3 CP Metam Sodium 51% SL_B-8.2).

The application parameters used for PEC_{SOIL} calculations are shown in Table 2.8.6-2.

Table 2.8.6-3: Input parameters related to application for PEC_{SOIL} calculations

Use No.	**1**	**2**
Crop	Bare soil (before planting of potato, carrot or onion)	Bare soil (before planting of capsicum pepper)
Application rate (kg as/ha)	153 kg/ha metam sodium 86.6 kg/ha MITC 3.52 kg/ha DMTU	306 kg/ha metam sodium 173.2 kg/ha MITC 7.04 kg/ha DMTU
Number of applications/interval	1	1
Crop interception (%)	0	0
Depth of soil layer (cm)	20 cm (incorporation)	5 cm (drip irrigation)*

* It should be noted that soil is worked after TIF sheeting is removed, which will re-distribute any remaining residue into lower soil depths. The standard 5 cm depth is used as a worst case.

The endpoints used for the risk assessment are summarised in Table 2.8.6-4.

Table 2.8.6-4: Substance parameters related to application for PEC_{SOIL} calculations

Substance	**Metam sodium**	**MITC**	**DMTU**
Soil DT_{50} (persistence endpoint)	0.0118 d (17 min)	3.24 d (at 153 kg metam sodium/ha) 5.0 d (at 306 kg metam sodium/ha)	0.308 d (7.39 h)*

* DMTU degrades with DFOP kinetics in the worst-case soil. The DFOP parameters are k_1=2.887 d^{-1}, k_2=0.009768 d^{-1}, g=0.848

Permanent greenhouse
It concerned the product Metam Na 510 SL (see Vol.3 CP Metam Na 510 SL_B-8.2).

According to the EFSA guidance for assessing environmental exposure following the use of plant protection products in greenhouses in the EU (EFSA, 2014a)[25]. A risk assessment for soil organisms in permanent greenhouses is only required for persistent substances (DT_{90} > 1 year). Since neither metam or MITC are considered persistent, no exposure or risk assessment is required for the uses on permanent greenhouse according to EFSA (2014).

[25] EFSA, 2014a. EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments. EFSA Journal 2014;12(3):3615, 43 pp., doi:10.2903/j.efsa.2014.3615.

Nevertheless, exposure to soil organisms outside the permanent greenhouses is possible via volatilisation from permanent greenhouses and deposition. This exposure pathway was taking into account in the risk assessment of the product Metam Na 510 SL (see Vol. 3 CP_ Metam Na 510 SL_B-8.2).

In separate studies performed by [redacted] (2020a), [redacted] (2020) and [redacted] (2020b) (see Vol. CP_ Metam Na 510 SL_B-8.5/01, 02, 03), the potential for redeposition of MITC outside of the greenhouse following use of metam-sodium was investigated. The maximum concentrations and corresponding redeposition are summarised in Table 2.8.6-5.

Table 2.8.6-5: Maximum concentrations and corresponding redeposition loading calculated for MITC

Redeposition Study Location	Max MITC Level (µg/L)	Volume of Water in Tray (L)	Surface Area of Tray (m^2)	MITC Redeposition (mg/m^2)
Spain, Spring	0.341	47.5	0.360	0.0450
Spain, Autumn	1.200	22.4	0.320	0.0840
Belgium, Autumn	0.780	32.7	0.345	0.0741

PEC_{SOIL} values for MITC *via* redeposition outside of the greenhouse were calculated assuming the maximum redeposition loading of 0.084 mg/m^2 (equivalent to 0.84 g/ha). As a conservative assumption, no plant interception was considered. Soil concentrations were calculated using standard assumptions with respect to soil density (1.5 g/cm^3) and incorporation depth (5 cm).
The decline of residues in soil using the worst-case laboratory soil DT_{50} value for MITC of 2.99 days ([redacted], 2020a), and simulations were run for 100 days. Calculated MITC residues constituted the instantaneous PEC_{SOIL} value at any given time.

Potential risk from redeposition of the impurity DMTU to soil outdoors following metam application in greenhouses is covered by the MITC risk assessment and therefore no DMTU PEC values are required. The risk assessment for MITC is worst case compared to DMTU because levels of DMTU would be lower than MITC (formed from metam) together with lower volatility and toxicity of DMTU in comparison to MITC.

The summary of PEC_{SOIL} calculated for the metam sodium products are presented in Table 2.8.6-6.

Table 2.8.6-6: Maximum PEC_{SOIL} after application of Metam Sodium products.

Crop	Application rate (kg a.s./ha)	Maximum PECs		
		Metam	MITC	DMTU
Permanent greenhouse (lettuce, ornamentals or baby leaf)	153 kg/ha metam sodium 86.6 kg/ha MITC	-	1.12E-03	-
Field (potatoes, carrots or onions)	153 kg/ha metam sodium 86.6 kg/ha MITC 7.04 kg/ha DMTU	51 000	28 867	1 173
Walk-in tunnels (peppers)	306 kg/ha metam sodium 173.2 kg/ha MITC 7.04 kg/ha DMTU	408 000	230 933	9 387
Greenhouse (before planting peppers)*	306 kg/ha metam sodium 173.2 kg/ha MITC 7.04 kg/ha DMTU	Not required	Not required	Not required

* The exposure to soil organisms outside the permanent greenhouses via volatilisation from permanent greenhouses and deposition was not taking into account for the product Metam Sodium 51% SL.

Ground water

Metam sodium is not relevant in groundwater since degradation to MITC occurs extremely rapidly (DT_{50} 4-17 min). MITC and DMTU were both modelled as parent compounds.

Field and walk-in tunnels
It concerned the product Metam Sodium 51% SL (see Vol.3 CP Metam Sodium 51% SL_B-8.3).

The predicted environmental concentrations in groundwater (PEC_{GW}) of MITC were assessed through simulations using the environmental fate models FOCUS-PEARL (v4.4.4), FOCUS-PELMO (v5.5.3) and MACRO-in-FOCUS (v5.5.4) in accordance with the requirements of European Regulations (EC) No 1107/2009 and (EU) No 284/2013. This assessment was performed according to the recommendations of the FOCUS Groundwater Scenarios Workshop and EFSA.

Modelling was conducted for outdoor applications of 153 kg/ha metam sodium to potatoes, carrots and onions using soil incorporation and for protected applications (walk-in tunnel) of 306 kg/ha metam sodium to fruiting vegetables using drip irrigation. The PEC in groundwater was determined as the 80th percentile of the annual average leaching concentrations at a depth of 1m, over a 20 year period. This was compared to the EU drinking water limit of 0.1 µg/L. The parameters related to the application, to MITC and to DMTU are summarised in Table 2.8.6-7, Table 2.8.6-8 and Table 2.8.6-9 respectively.

Table 2.8.6-7: Input parameters related to application for PEC_{GW} calculations

Crop	**Potato, Carrot, Onion**	**Tomato**
Application rate (kg MITC/ha)	0.0846	1.832
Application rate (kg DMTU/ha)	3.519	7.038
Number of applications/interval (d)	1	1
Relative application date	No	No
Crop interception (%)	0	0
Soil incorporation (m)	0.2	0
Frequency of application	annual	annual
Models used for calculation	FOCUS PEARL v4.4.4, FOCUS PELMO v5.5.3	FOCUS PEARL v4.4.4, FOCUS PELMO v5.5.3, MACRO-in-FOCUS v5.5.4

Table 2.8.6-8: Input parameters related to MITC for PEC_{GW} calculations

Input Parameter	**MITC**	**Reference**
Molecular weight (g/mol)	73.12	EFSA (2011)
Water solubility (mg/l)	8940	EFSA (2011)
Saturated vapour pressure (Pa)	1739 at 20°C	EFSA (2011)
DT_{50} in soil (d) (outdoor use)	2.1 (geomean normalisation to pF2, 20 °C with Q_{10} of 2.58, n =4)	EFSA (2015)
DT_{50} in soil (d) (indoor use)	3.2 (geomean normalisation to pF2, 20 °C with Q_{10} of 2.58, n =4)	EFSA (2015)
K_{foc} (mL/g)/K_{fom}	21.7/12.6 (geomean, n = 10)	EFSA (2011) + [redacted] (2010) $K_{fom} = K_{foc}$ x 0.58
1/n	0.86 (arithmetic mean, n = 10)	EFSA (2011) + [redacted] (2010)
Plant uptake factor	0	Default

Table 2.8.6-9: Input parameters related to DMTU for PEC_{GW} calculations

Input Parameter	**DMTU**	**Reference**
Molecular weight (g/mol)	104.18	EFSA (2011)
Water solubility (mg/L)	832.95	EFSA (2011)

Input Parameter	DMTU	Reference
Saturated vapour pressure (Pa)	581980 at 20°C	EFSA (2011)
DT_{50} in soil (d)	0.202 (geomean normalisation to pF2, 20 °C with Q_{10} of 2.58, n =8)	EFSA (2011) + [redacted] (2011) + [redacted] (2011)
K_{foc} (mL/g)/K_{fom}	8.1/4.7 (geomean, n = 9)	EFSA (2011) + [redacted] (2010) $K_{fom} = K_{foc}$ x 0.58
1/n	0.80 (arithmetic mean, n = 9)	EFSA (2011) + [redacted] (2010)
Plant uptake factor	0	Default

The presence of TIF prevents water infiltration, which prevents leaching, and also prevents volatile losses of MITC. Therefore, the risk for groundwater will occur after the TIF is removed, when the soil is exposed to conditions may result in leaching. The application rate of MITC was therefore reduced according to the degradation of MITC over 21 days using standard SFO kinetics. The soil DT_{50} of 2.1 days or 3.2 days (for outdoor and protected uses, respectively) was used to calculate an equivalent application rate, as follows:

Application rate (outdoor) = $86600\ e^{(-21 * \ln(2) / 2.1)} = 84.6$ g/ha

Application rate (walk-in tunnel) = $173200\ e^{(-21 * \ln(2) / 3.2)} = 1832$ g/ha

Applications in March, April, September and October were modelled to represent a suitable range of worst-case conditions. Application was set for the 1st of each month for all crops, so that crop and climate effects can be compared.

PEC_{GW} values were below the 0.1 µg/L limit for MITC in all crops and scenarios for the months of March, April, September and October, using PELMO 5.5.3 and PEARL 4.4.4 for outdoor application on potatoes, carrots and onions.

PEC_{GW} values were below the 0.1 µg/L limit for MITC for indoor application on tomatoes in all scenarios for the months of March, April, September and October, using PELMO 5.5.3 and MACRO 5.5.4. When using PEARL 4.4.4, PEC_{GW} values were below the 0.1 µg/L in all scenarios and timings except the October Piacenza scenario, where the PEC_{GW} was 0.174959 µg/L.

A relevance assessment for the metabolite MITC is therefore necessary (see point 2.11).

PEC_{GW} values were below the 0.1 µg/L limit for DMTU in all crops and scenarios for the months of March, April, September and October, using PELMO 5.5.3 and PEARL 4.4.4 for outdoor application on potatoes, carrots and onions, except for the March simulation of the Jokioinen scenario. The Jokionen scenario uses climate data for Northern Europe and the March soil temperatures are below the GAP requirement of 10°C. It therefore does not represent a situation where metam sodium could be applied.

PEC_{GW} values were below the 0.1 µg/L limit for DMTU for indoor application on tomatoes in all scenarios for the months of March, April, September and October, using PELMO 5.5.3, PEARL 4.4.4 and MACRO 5.5.4.

Permanent greenhouse
It concerned the product Metam Na 510 SL (see Vol.3 CP Metam Na 510 SL_B-8.3).

A series of higher tier greenhouse scenarios were developed, for use with FOCUS-PEARL 4.4.4, to simulate triennial, pre-planting use of metam on leafy vegetables and ornamentals *via* drip irrigation under TIF. The approach used in this study is in line with the approach and appendices outlined in the EFSA protected crops guidance (EFSA, 2014).

The scenarios developed in this study are based on the FOCUS Piacenza scenario and the FOCUS cabbage crop, which was used to represent both leafy vegetables and ornamentals. A series of bespoke Piacenza weather files were built, accounting for the difference in average daily temperature and solar radiation inside a greenhouse as described for the EFSA site at Pistoia, Italy. Humidity and wind speeds within the greenhouse were also modified in line with literature values. Irrigation volumes were calculated using PEARL and applied as rainfall, with an additional rainfall event (6 mm) included in the weather file to represent metam application *via* drip irrigation. Following application, no irrigation was applied for a six-week period while the TIF cover was in place. No further alterations were made to the weather files to reflect the presence of the TIF cover, e.g. to modify the temperature underneath the plastic.

Both MITC and DMTU were modelled as parent compounds, with applications simulated every three years. A wide range of application timings are possible for the product, therefore 12 separate scenarios were constructed to investigate metam use in each month of the year, with application simulated on the first day of the corresponding month (1st Jan or 1st Feb etc.). With the exception of the six-week period following the application date (i.e. when the TIF was in place), the cabbage crop was present throughout the year, and a corresponding crop calendar and weather file was constructed for each scenario. The presence of the crop throughout most of the year leads to a greater groundwater recharge, as irrigation is applied only when the crop is growing, and can therefore be considered to provide a conservative assessment of exposure.

The chemical input parameters used in the modelling for MITC and DMTU were taken from the EFSA Conclusion for metam (EFSA, 2011) and are summarised in Table 2.8.6-10. K_{foc} values were derived as the geometric means of the available data, in accordance with the latest guidance from EFSA. To suppress volatilisation in the modelling, in line with the use of gas-tight TIF covers, the boundary air layer thickness was increased from 0.01 m (FOCUS default) to 1 m (the maximum possible value in PEARL), and the diffusion coefficients of MITC and DMTU in air were reduced from the FOCUS default of 0.43 m^2/day to 0.1 m^2/day (the lowest value PEARL will accept).

Table 2.8.6-10: Chemical input parameters used in the modelling

Property	MITC	DMTU	Reference
Molar mass (g/mol)	73.12	104.18	EFSA, 2011
Solubility in water, 20°C (mg/L)	8940	581980	EFSA, 2011
Vapour pressure, 20°C (Pa)	1739	832.95	EFSA, 2011
K_{foc} (mL/g)	13.0 (n=5)	8.4 (n=4)	Geometric mean calculated from K_{foc} values in EFSA 2011; no pH dependence
K_{fom} (mL/g)	7.5	4.9	$K_{om} = K_{oc}/1.724$ (FOCUS, 2014)
1/n	0.83 (n=5)	0.76 (n=4)	EFSA 2011
DT_{50} in soil, 20°C, pF2 (d)	2.1 [a] (n=5)	0.121 (n=4)	EFSA 2011
Plant uptake factor	0	0	Non-systemic FOCUS default

a – DT_{50} value appropriate for a maximum MITC application rate of 86.6 kg/ha (max 153 kg metam-Na/ha; EFSA, 2011).

Predicted environmental concentrations of MITC and DMTU in groundwater (PEC_{GW}), as represented by the 80th percentile period average concentrations at a depth of 1 m, were <0.001 µg/L for all application dates simulated.

Additional simulations were performed taking into account movements of MITC in the gas phase of soil during the TIF cover period.

Four field soil dissipation studies were conducted in different locations across Europe including Spain, Italy, Hungary and Germany. MITC residues in the 0 – 90 cm soil horizons were analysed up to 42 DAA (days after application) when the TIF cover was removed. For the field dissipation studies conducted in Spain, Italy and Hungary, MITC residues were not detected in 30 – 90 cm soil horizons after 14 DAA ([redacted], 2020a, b; Vol. 3 CA B.8.1.1.2.2.1/05, 06). During the whole trial period for the field study in Germany ([redacted], 2020c; Vol. 3 CA B.8.1.1.2.2.1/07), MITC predominantly resided in the upper 0 – 40 cm layers accounting between 99.7 and 100.0% (average values) of the amounts recovered in the soil profile at each sampling event. In the upper 0 – 40 cm horizon, trace amounts between <LOQ and 0.30 mg/kg dry soil from 21 DAA until last sampling at 13 DAR were found. From 21 DAA to 13 DAR, majority of the results indicated that MITC was not detected in the 40 – 90 cm soil layer, with only 6 detections below the LOQ (one at 21 DAA, three at 42 DAA, one at 3 DAR and one at 13 DAR.

Based on the detectable residues, a maximum equivalent application rate of 3.58 – 53.1 g/ha was calculated for each soil horizon (0 – 90 cm) from MITC residues (mg/kg) measured on 42 DAA for the field dissipation study in Germany ([redacted], 2020c; Vol. 3 CA B.8.1.1.2.2.1/07). Separate groundwater simulations were performed for each soil horizon based on injection application method at a specified soil depth, as summarised in Table 2.8.6-11.

Table 2.8.6-11: Effective MITC application rates and soil depths used for groundwater (PEC_{GW}) simulations based on sampling on DAA 42 for the German field dissipation study

Soil Horizon (cm)	Density (g/cm^3)	Replicate 1[a]		Replicate 2[a]		Replicate 3[a]		Appl Rate (g/ha)[b]	Soil Depth for Injection
		(mg/kg)	(g/ha)	(mg/kg)	(g/ha)	(mg/kg)	(g/ha)		
0 - 10	1.607	0.00837	13.5	0.03306	53.1	0.02877	46.2	**53.1**	**10 cm**
10 - 20	1.607	0.02264	36.4	0.01488	23.9	0.01224	19.7	**36.4**	**20 cm**
20 - 30	1.607	0.00432	6.95	0.00466	7.49	0.00435	6.99	**7.49**	**30 cm**
30 - 40	1.588	<LOD	<LOD	0.00329	5.22	0.00328	5.21	**5.22**	**40 cm**
40 - 50	1.588	<LOD	<LOD	0.00297	4.71	0.00300	4.76	**4.76**	**50 cm**
50 - 60	1.588	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	n.a
60 - 70	1.588	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	n.a
70 - 80	1.588	<LOD	<LOD	<LOD	<LOD	0.00225	3.58	**3.58**	**80 cm**
80 - 90	1.588	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	n.a
0 - 90	-	**0.035**	**56.8**	**0.059**	**94.5**	**0.054**	**86.5**	**94.5**	n.a

[a]MITC residues for DAA 42 from Germany field dissipation study ([redacted], 2020c; Vol. 3 CA B.8.1.1.2.2.1/07).
[b]Maximum application rate (g/ha) for three replicates. g/ha = conc. (mg/kg) x 100 x depth of soil layer (10 cm) x soil layer density (g/cm^3)
LOQ of the analytical method was approximately 0.01 mg/kg for MITC, with the LOD set at 0.002 mg/kg (20% of LOQ).
n.a: Not applicable.

The same parameters than first calculations were used for these additional simulation except the DT_{50} value. A DT_{50} value of 2.99 days was used for the additional PEC_{GW} simulations (highest DT50 value from the field dissipation studies).

Calculated PEC_{GW} were <0.001 µg/L for MITC residues present in soil horizons 0 – 50 cm. The highest PEC_{GW} were calculated for MITC residues present in the 70 – 80 cm soil horizon. The PEC_{GW} calculated by combining all soil horizons ranged from 0.004 – 0.041 µg/L. This range is less than the parametric drinking water limit of 0.1 µg/L for all simulations.

The summary of PEC_{GW} calculated for the metam sodium products are presented in Table 2.8.6-12.

Table 2.8.6-12: Maximum PEC_{GW} after application of Metam Sodium products.

Crop	Application rate (kg a.s./ha)	Maximum PEC_{GW} (µg/L)	
		MITC	DMTU
Permanent greenhouse (lettuce, ornamentals or baby leaf)	153 kg/ha metam sodium 86.6 kg/ha MITC 7.04 kg/ha DMTU	Piacenza adapted to permanent greenhouse: 0.041	<0.001
Field (potatoes, carrots or onions)*	153 kg/ha metam sodium 86.6 kg/ha MITC 7.04 kg/ha DMTU	<0.001	<0.001
Greenhouse and walk-in tunnels (peppers)*	306 kg/ha metam sodium 173.2 kg/ha MITC 7.04 kg/ha DMTU	Châteaudun : 0.006 **Piacenza: 0.175** Porto: 0.012 Sevilla: <0.001 Thiva: <0.001	<0.001

* The movements of MITC in the gas phase of soil during the TIF cover period was not taking into account for the product Metam Sodium 51% SL.

Surface water and sediment

Metam sodium is not relevant in surface water since degradation to MITC occurs extremely rapidly (DT_{50} 4-17 min).

Field
It concerned the product Metam Sodium 51% SL (see Vol.3 CP Metam Sodium 51% SL_B-8.2).

The predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) of MITC were assessed through simulations using the environmental fate model FOCUS-STEPS 1-4 in accordance with the requirements of European Regulations (EC) No 1107/2009 and (EU) No 284/2013. The PEC was modelled using the STEPS 1-2 calculator (version 3.2), FOCUS-SWASH (version 5.3) and SWAN (version 5.0.0), following the recommendations of the FOCUS Working Group on Surface Water Scenarios. Mitigation measures were modelled according to the recommendation of the FOCUS Working Group on Landscape and Mitigation Factors.

Modelling was conducted for drip irrigation application of metam sodium at 153 kg/ha to potatoes and the four FOCUS vegetable crop groups (bulb, fruiting, leafy, root).

A summary of the environmental fate parameters is given in Table 2.8.6-13.

Table 2.8.6-13: Input parameters related to MITC for $PEC_{sw/sed}$ calculations

Parameter	**Value (MITC)**	**Reference**
Molecular weight (g/mol)	73.12	EFSA (2011)
Saturated vapour pressure (Pa)	1739 (at 20°C)	EFSA (2011)
Water solubility (mg/L)	8940 (at 20°C)	EFSA (2011)
Diffusion coefficient in water (m^2/d)	4.3×10^{-5}	Default
Diffusion coefficient in air (m^2/d)	0.43	Default
K_{foc} (mL/g)	21.7 (geometric mean, n = 10)	EFSA (2011) + [redacted] (2010) CA 7.1.3.1.2/01
Freundlich Exponent 1/n	0.86 (arithmetic mean, n = 10)	EFSA (2011) + [redacted] (2010) CA 7.1.3.1.2/01
Plant Uptake	0	Worst-case default
Wash-Off factor from Crop (1/mm)	0.05 (MACRO) 0.50 (PRZM)	Default
$DT_{50,soil}$ (d)	2.1 (20 °C / pF2, geomean n = 4)	EFSA (2011)
$DT_{50,water}$ (d)	55.8 (20°C, geomean n = 2)	[redacted] (2013) CA 7.2.2.3/01
$DT_{50,sed}$ (d)	STEP 2: 55.8 (20°C, geomean n = 2) STEP 3-4: 1000	[redacted] (2013) CA 7.2.2.3/01 Worst-case default
$DT_{50,whole\ system}$ (d)	STEP 1: 55.8 (20°C, geomean n = 2)	[redacted] (2013) CA 7.2.2.3/01

The presence of TIF prevents water infiltration and volatilisation, which prevents entry into water bodies via drainflow, runoff or volatile deposition. Therefore, aquatic exposure will occur after the TIF is removed, when the soil is exposed to conditions that may result in drainflow, runoff or volatile deposition to surface water. The application rate of MITC was therefore reduced according to the degradation of MITC over 21 days using standard SFO kinetics:

$$\text{Application rate} = 86600\ e^{(-21 * \ln(2) / 2.1)} = 84.6 \text{ g/ha}$$

At STEPS 3 and 4, the crop type must be selected even when no crop is present at the time of application. Applications were simulated for each crop, with separate runs for March, April, September and October to represent a range of common application timings (application windows starting on the 1st of each month and extending for 30 days). All crops were modelled as a drip irrigation treatment, since this is the worst-case compared to soil incorporation. STEP 4 models were used to apply runoff mitigation using 20m Vegetated Filter Strips (VFS). Volatile deposition is difficult to quantitatively assess, and reliable experimental data were not yet available. As a worst-case estimate, the maximum values for volatile pesticides were applied using the German EVA spreadsheet tool.

PEC_{sw} values at STEPS 1-2 were relatively high, due to the simplistic assumptions used in the models. At STEP 3 the crop type generally had little effect on the PEC_{sw}, with some exceptions where the application date was shortly before emergence or after harvest. The PEC_{sw} values will generally overestimate runoff since they assume undisturbed fallow soil, which will have higher runoff curve numbers than the recently worked soil following removal of TIF. Nevertheless, the worst-case PEC_{sw} values are considered suitable for a highly conservative risk assessment. A 20 m vegetated filter strip was modelled in order to reduce the runoff loadings, and the STEP 4 PEC_{sw} for the worst-case crop in each scenario and month are given below in Table 2.8.6-14. All PEC values are suitable for use in ecotoxicological risk assessment.

Table 2.8.6-14: Worst-case FOCUS STEP 4 PEC_{sw} for MITC in each month (20m VFS and volatile deposition)

FOCUS Scenario	Waterbody (Crop number)	Maximum PEC_{sw} (µg/L)				
		Mar	Apr	Sep	Oct	Overall Max
D3	ditch (1)	0.02084	0.01939	0.01467	0.01473	0.02084
D3	ditch (2)	0.02079	0.01936	n/r	n/r	0.02079
D4	pond (1)	0.006003	0.005893	0.004728	0.005121	0.006003
D4	stream (1)	0.006715	0.005091	0.007784	0.008400	0.008400
D6	ditch (1)	0.02087	0.03110	0.01253	0.01400	0.03110
D6	ditch (2)	0.01848	0.03110	0.01253	0.01400	0.03110
R1	pond (1)	0.005866	0.005381	0.004492	0.005216	0.005866
R1	pond (2)	0.005866	0.005381	n/r	n/r	0.005866
R1	stream (1)	0.07804	0.02618	0.2083	0.1995	0.2083
R1	stream (2)	0.07739	0.07126	n/r	n/r	0.07739
R2	stream (1)	0.02276	n/r	0.008957	0.04382	0.04382
R2	stream (2)	0.02278	0.009104	n/r	n/r	0.02278
R3	stream (1)	0.3702	0.2407	0.007711	0.03813	0.3702
R3	stream (2)	0.3689	0.1398	n/r	0.01192	0.3689
R4	stream (1)	0.1401	0.03302	0.009029	0.3569	0.3569
R4	stream (2)	0.1401	0.08754	0.009029	0.2892	0.2892

n/r = not relevant because no modelled crops have bare soil during this application timing

Walk-in tunnels

It concerned the product Metam Sodium 51% SL (see Vol.3 CP Metam Sodium 51% SL_B-8.2).

The predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) of MITC were assessed through simulations using the environmental fate model FOCUS-STEPS 1-3 in accordance with the requirements of European Regulations (EC) No 1107/2009 and (EU) No 284/2013. The PEC was modelled using the STEPS 1-2 calculator (version 3.2) and FOCUS-SWASH (version 5.3), following the recommendations of the FOCUS Working Group on Surface Water Scenarios and the EFSA (2014) guidance on emissions from protected crops.

Modelling was conducted for drip irrigation application of metam sodium at 306 kg/ha to leafy and fruiting vegetables, in order to provide a suitable range of application timings and scenarios to cover potential uses conditions. Input parameters for MITC were taken from the EU agreed endpoints from the previous EU review (including post-approval confirmatory data), combined with new data submitted by Lainco S.A (summarised in the active substance dossier. To model the application scheme, MITC was treated as an active substance since it is formed extremely rapidly from metam (EFSA DT_{50} = 4-17 minutes). The presence of TIF prevents water infiltration and volatilisation, which prevents entry into water bodies via drainflow or volatile deposition. Therefore, aquatic exposure will occur after the TIF is removed, when the soil is exposed to conditions that may result in drainflow, runoff or volatile deposition to surface water. The application rate of MITC was therefore reduced according to the degradation of MITC over 21 days using standard SFO kinetics:

$$\text{Application rate} = 173200\ e^{(-21 * \ln(2) / 3.2)} = 1832 \text{ g/ha}$$

A summary of the environmental fate parameters is given in Table 2.8.6-13.

Table 2.8.6-13: Input parameters related to MITC for $PEC_{sw/sed}$ calculations

Parameter	Value (MITC)	Reference
Molecular weight (g/mol)	73.12	EFSA (2011)
Saturated vapour pressure (Pa)	1739 (at 20°C)	EFSA (2011)
Water solubility (mg/L)	8940 (at 20°C)	EFSA (2011)
Diffusion coefficient in water (m^2/d)	4.3×10^{-5}	Default
Diffusion coefficient in air (m^2/d)	0.43	Default
K_{foc} (mL/g)	21.7 (geometric mean, n = 10)	EFSA (2011) + [redacted] (2010) CA 7.1.3.1.2/01
Freundlich Exponent 1/n	0.86 (arithmetic mean, n = 10)	EFSA (2011) + [redacted] (2010) CA 7.1.3.1.2/01
Plant Uptake	0	Worst-case default
Wash-Off factor from Crop (1/mm)	0.05 (MACRO) 0.50 (PRZM)	Default
$DT_{50,soil}$ (d)	3.2 (20 °C / pF2, geomean n = 4)	EFSA (2011)
$DT_{50,water}$ (d)	55.8 (20°C, geomean n = 2)	[redacted] (2013) CA 7.2.2.3/01
$DT_{50,sed}$ (d)	STEP 2: 55.8 (20°C, geomean n = 2) STEP 3-4: 1000	[redacted](2013) CA 7.2.2.3/01 Worst-case default
$DT_{50,whole\ system}$ (d)	STEP 1: 55.8 (20°C, geomean n = 2)	[redacted] (2013) CA 7.2.2.3/01

At STEP 3, the crop type potentially affects the modelled scenarios even when no crop is present at the time of application. Applications were simulated for leafy and fruiting vegetable crops, with separate runs for March, April, September and October to represent a range of common application timings (application windows starting on the 1st of each month and extending for 30 days). Volatile deposition was not modelled, since reliable data were not available for emissions from protected uses. Runoff scenarios were not modelled as they are not relevant to walk-in tunnels.

PEC_{sw} values at STEPS 1-2 were relatively high, due to the simplistic assumptions used in the models. The PEC_{sw} values at STEP 3 are summarised in Table 2.8.6-15. At STEP 3 the crop type affected the PEC_{sw} in the D6 scenario during April, since crop irrigation is applied shortly after application to fruiting vegetables but not to leafy vegetables. Irrigation will not be applied shortly after removal of TIF when potentially phytotoxic MITC residues prevent planting of crops, therefore the leafy vegetable scenario is considered more relevant for all crop types.

The October simulations resulted in high drainflow in some scenarios, due to high levels of rainfall. These were not considered representative of the use of metam sodium, as little or no irrigation will be applied at this time to the bare soil in protected walk-in tunnel conditions.

The PEC_{sw} and PEC_{sed} values are suitable for use in environmental risk assessment but it should be noted that they represent extreme worst-case conditions. No FOCUS surface water scenarios have been developed for protected uses and field rainfall data must be used. This results in a climate that is far more extreme than the controlled conditions in a walk-in tunnel.

Table 2.8.6-15: Peak FOCUS STEP 3 PEC_{sw} for MITC in protected crops

FOCUS Scenario	Waterbody	Crop	Maximum PEC_{sw} (µg/L)				
			March	April	Sept.	Oct.	Max.
D3	ditch	Leafy vegetables (early crop)	<0.000001	<0.000001	0.000085	0.002076	0.002076
D3	ditch	Leafy vegetables (late crop)	<0.000001	<0.000001	crop present	crop present	<0.000001
D4	pond	Leafy vegetables	0.000063	0.000023	crop present	0.1948	0.1948

D4	stream	Leafy vegetables	0.001633	0.000380	crop present	0.8948	0.8948
D6	ditch	Leafy vegetables	0.2997	0.04698	crop present	crop present	0.2997
D6	ditch	Fruiting vegetables	0.2968	1.150	0.000300	0.8407	1.150

The PEC in surface water and sediment was determined for the impurity DMTU, following application of metam sodium at 306 kg/ha via drip irrigation (the worst-case use in the GAP). The PEC was modelled according to FOCUS guidelines, using the STEPS 1-2 calculator (version 3.2).

Input parameters for DMTU are given in Table 2.8.6-16. for the reapproval of the active substance metam. Application was modelled using the worst-case DMTU application rate of 7.038 kg/ha. It should be noted that typical levels of DMTU will be much lower than this maximum value. Applications were modelled for all available STEP 2 locations and application timings.

Table 2.8.6-16: Input parameters related to DMTU for $PEC_{sw/sed}$ calculations

Parameter	Value (DMTU)	Reference
Molecular weight (g/mol)	104.18	EFSA (2011)
Water solubility (mg/L)	581980 (at 20°C)	EFSA (2011)
K_{foc} (mL/g)	8.1 (geometric mean, n = 9)	EFSA (2011) + [redacted] (2011)
$DT_{50,soil}$ (d)	0.202 (20 °C / pF2, geomean n = 8)	EFSA (2011) + [redacted] (2011) + [redacted] (2011)
$DT_{50,water}$ (d)	1000	Worst-case default
$DT_{50,sed}$ (d)	1000	Worst-case default
$DT_{50,whole\ system}$ (d)	1000	Worst-case default

As an extreme worst-case, the application of DMTU has been modelled without considering any mitigating effect from the TIF covering. In reality, the use of TIF sheeting will allow a high level of soil degradation while preventing conditions that cause drainflow or runoff or volatile loss, as rainwater cannot penetrate the sheeting.

The PEC values for STEPS 1-2 are summarised in Table 2.8.6-17. The PEC_{sw} and PEC_{sed} values are suitable for establishing an exposure envelope for environmental risk assessment.

Table 2.8.6-17: Summary of PEC Values for DMTU at STEPS 1-2

FOCUS STEP and Scenario	Season	Max PEC_{sw} (µg/L)	TWA 21d - PEC_{sw} (µg/L)	Max PEC_{sed} (µg/kg)
STEP 1	-	2320	2300	188
STEP 2				
Northern Europe	March-May	0.0004	0.0004	<0.0001
	Jun-Sep	0.0004	0.0004	<0.0001
	Oct-Feb	0.0011	0.0011	0.0001
Southern Europe	March-May	0.0009	0.0009	0.0001
	Jun-Sep	0.0007	0.0007	0.0001
	Oct-Feb	0.0009	0.0009	0.0001

Permanent greenhouse
It concerned the product Metam Na 510 SL (see Vol.3 CP Metam Na 510 SL_B-8.3).

Simulations were performed using the SWASH 5.3 interface, with the FOCUS-MACRO 5.5.4 and FOCUS-TOXSWA 5.5.3 models. A series of higher tier greenhouse scenarios was developed, for use with FOCUS-MACRO 5.5.4 and FOCUS-TOXSWA 5.5.3, to assess the potential for MITC to reach surface water *via* drainage following the use of metam with TIF on leafy vegetables and ornamentals in greenhouses. In a subsequent step, surface water exposure *via* redeposition of MITC was also assessed, using redeposition loadings derived from three experimental studies ([redacted], 2020a,b; [redacted], 2020).

In these studies, water trays were placed in eight directions around a greenhouse that was treated with metam, at distances of 10 m, 20 m and 30 m from the greenhouse. The water contained in the trays was sampled at regular intervals to quantify MITC residues. A maximum concentration of 1.20 µg/L MITC was detected 24 hours after application, in a tray 10 m from the greenhouse, corresponding to an effective redeposition loading of 0.084 mg/m^2. This loading was entered into the TOXSWA input (.txw) files on the day of application as a drift loading, with an additional 20% contribution included for stream water bodies to represent treatment of the upstream catchment.

The drainage scenarios developed in this study are based on the FOCUS D4 leafy vegetables scenario, which was used to represent both leafy vegetables and ornamentals. A series of bespoke D4 weather files was built, accounting for the difference in average daily temperature and solar radiation inside a greenhouse as described for the EFSA site at Pistoia, Italy. Humidity and wind speeds within the greenhouse were also modified in line with literature values. Irrigation volumes were calculated from daily evapotranspiration values output by the MACRO model, with an additional rainfall event (6 mm) included in the weather file to represent metam application *via* drip irrigation, and these volumes were applied in the modelling as rainfall. Following application, no irrigation was applied for a six-week period while the TIF cover was in place. No further alterations were made to the weather files to reflect the presence of the TIF cover, e.g. to modify the temperature underneath the plastic.

MITC was simulated as a parent compound at an application rate of 86.6 kg/ha, assuming 100% transformation instantaneously from metam. A wide range of application timings are possible for the product, therefore 12 separate scenarios were constructed to investigate metam use in each month of the year, with application simulated on the first day of the corresponding month (1st Jan or 1st Feb etc.). With the exception of the six-week period following the application date (i.e. when the TIF was in place), the leafy vegetables crop was present throughout the year, and a corresponding crop calendar and weather file were implemented for each scenario.

Input parameters for MITC are given in Table 2.8.6-18.

Table 2.8.6-18: Chemical input parameters used in the modelling

Property	MITC	Reference
Molar mass (g/mol)	73.12	EFSA, 2011
Solubility in water, 20°C (mg/L)	8940	EFSA, 2011
Vapour pressure, 20°C (Pa)	1739	EFSA, 2011
K_{foc} (mL/g)	13.0	Geometric mean calculated from K_{foc} values in EFSA 2011; no pH dependence (n=5)
K_{fom} (mL/g)	7.5	$K_{om} = K_{oc}/1.724$ (FOCUS, 2014)
1/n	0.83	EFSA 2011 (n=5)
DT_{50} in soil, 20°C, pF2 (d)	2.1 [a]	EFSA 2011 (n=5)
DT_{50} in water, 20°C (d)	55.8	[redacted] 2013 (n=2)
DT_{50} in sediment, 20°C (d)	1000	FOCUS default
Plant uptake factor	0	Non-systemic FOCUS default
Washoff coefficient (mm^{-1})	0.05	FOCUS default
Foliar DT_{50} (d)	10	FOCUS default

a – DT_{50} value appropriate for a maximum MITC application rate of 86.6 kg/ha (max 153 kg metam-Na/ha; EFSA, 2011).

Maximum PECsw and PECsed for MITC, as output by the FOCUS-TOXSWA 5.5.3 model, are given in the Table 2.8.6-19.

Table 2.8.6-19: Predicted maximum environmental concentrations of MITC in surface water and sediment following use of metam with TIF on leafy vegetables and ornamentals in greenhouse

Application date	MITC exposure *via* drainflow only	MITC exposure *via* drainflow + redeposition [a]

	PECsw (µg/L)	PECsed (µg/kg)	PECsw (µg/L)	PECsed (µg/kg)
Greenhouse scenario; pond waterbody				
Jan 1st	<0.001	<0.001	0.084	0.015
Feb 1st	<0.001	<0.001	0.084	0.015
Mar 1st	<0.001	<0.001	0.084	0.015
Apr 1st	<0.001	<0.001	0.084	0.015
May 1st	<0.001	<0.001	0.084	0.012
Jun 1st	<0.001	<0.001	0.084	0.010
Jul 1st	<0.001	<0.001	0.084	0.009
Aug 1st	<0.001	<0.001	0.084	0.009
Sep 1st	<0.001	<0.001	0.084	0.011
Oct 1st	<0.001	<0.001	0.084	0.012
Nov 1st	<0.001	<0.001	0.084	0.015
Dec 1st	<0.001	<0.001	0.084	0.015
Greenhouse scenario; stream waterbody				
Jan 1st	<0.001	<0.001	0.319	0.018
Feb 1st	<0.001	<0.001	0.321	0.019
Mar 1st	<0.001	<0.001	0.322	0.021
Apr 1st	<0.001	<0.001	0.323	0.021
May 1st	<0.001	<0.001	0.323	0.019
Jun 1st	<0.001	<0.001	0.318	0.015
Jul 1st	<0.001	<0.001	0.307	0.011
Aug 1st	<0.001	<0.001	0.301	0.010
Sep 1st	<0.001	<0.001	0.301	0.010
Oct 1st	<0.001	<0.001	0.306	0.012
Nov 1st	<0.001	<0.001	0.312	0.014
Dec 1st	<0.001	<0.001	0.316	0.016

[a]The redeposition loading used in the modelling was calculated based on a worst-case assumption of 0.084 mg/m^2 for pond waterbody and 0.101 mg/m^2 for stream waterbody

Other routes of exposure

Environmental exposure is possible via direct soil contamination and indirect transfer via drainflow, runoff, leaching or volatilisation to groundwater, surface water or air. These routes have been evaluated under the data points above. No other routes of exposure are expected. Thus, no additional estimations of concentrations are required.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1 Birds

Avian toxicity studies were conducted with Bobwhite quail and Mallard ducklings for either metam-sodium or the active metabolite MITC. A summary of the toxicity endpoints in shown in Table 2.9.1.1-1.

Table 2.9.1.1-1: Summary of avian endpoints for the active substance metam and its metabolite

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus)*	Metam-sodium	Single dose (Acute oral toxicity)	Oral LD_{50}	211 mg a.s./kg bw (equivalent to **119 mg MITC/kg bw**)	CA8.1.1.1/01 [redacted], 1985
Bobwhite quail (*Colinus virginianus*)	MITC	Single dose (Acute whole-body inhalation toxicity)	Inhalation LC_{50}	**127 ppm MITC (males)** 181 ppm MITC (females)	CA8.1.1.1/02 [redacted] 2012
Bobwhite quail (*Colinus virginianus*)	Metam-sodium	5 days (Short-term dietary toxicity)	Oral LC_{50}	> 5000 mg a.s./kg diet (> 448 mg a.s./kg bw/d)	CA8.1.1.2/01 [redacted] 1993a
Mallard ducklings (*Anas platyrhynchos*)	Metam-sodium	5 days (Short-term dietary toxicity)	Oral LC_{50}	> 5000 mg a.s./kg diet (> 324 mg a.s./kg bw/d) (equivalent to > **183.4 mg MITC/kg bw/d**)	CA8.1.1.2/02 [redacted] 1993b

*Note: **bold** – endpoint used for the current risk assessment*

Higher tier data intended to refine exposure estimates (such as residues in bird and mammal food items, focal species, body burden modelling investigation) for outdoor uses of metam were submitted.

Table 2.9.1.1-2: Summary of higher tier data submitted by the applicant Eastman (Taminco)

Higher tier data on birds
KCP 10.1.1.2/01 [redacted] **(2010). Bird censuses on sterilized and unsterilized carrot plots in Les Landes, France during spring 2008** [redacted] **Study Nr.** [redacted] With a few exceptions the bare cultivated land was definitely not a favoured habitat for most of the 74 bird species identified in and around the field and the frequency of bird visits remained low throughout the study period. Starling and Mistle thrush were observed to remove food items from the treated soil and consequently may have been exposed to contaminated food. In April Crows were regularly seen on the field, but to what extent they may have been exposed is unclear. Their shyness prevented detailed observations. The White wagtail was resident to the field but somehow restricted its presence to water treated plots. Swallows foraged low over the field, obviously eating insects emerging from the soil. To what extent these insects may have been contaminated is unknown. This study was evaluated during EU review and was considered acceptable for the identification of focal species (see EFSA Journal 2011; 9(9):2334 and PRAPeR 53). Agreed focal species for: - Insectivorous birds: Starling - Vermivorous birds: Mistle thrush

Higher tier data on birds

KCP 10.1.1.2/02

[redacted] (2011). Review of Field study [redacted] by [redacted] (2008) in the context of risk assessment for insectivorous birds potentially feeding on contaminated prey items after treatment with Metam-sodium

The values of PT derived from fixed-point observations and walked route observations using the number of individuals observed in treated plots as a proportion of those in all plots were 0.66 and 0.63 respectively. These remarkably similar values reduce the uncertainty regarding the influence of the census/walking routes and would represent a mean PT if all starlings fed exclusively on the treated fields. This is however an extreme overestimate of mean PT since the authors make it clear that the population from which the observed birds are drawn is considerably larger than the population seen only on the field itself. Although starlings were the most frequently seen insectivore in the April route walking observations they were only recorded on 5 % of the Metam-sodium plots observations.

The similarity of these two PT values suggests that the bias due to the observer in the walked route observations was minimal. In addition, the data show that overall very little feeding actually occurred on the treated field compared with that in the neighbouring habitats.

Could individual starlings be deriving all of their food from within the Metam-sodium treated plots? The EFSA (2009) guidance in section 6.1.5.6. requires a consideration of the range of PT for individual animals. Both the walked route and the fixed point observations contain days (12th and 14th April) with no observations at all in Metam-sodium treated plots interspersed with days (11th and 13th April) where they occurred. Even if the birds recorded as being seen in Metam-sodium treated plots were in fact the same individuals being seen repeatedly, assuming that similar dietary requirements are met each day, then the highest possible PT over the four day period following treatment would be 0.5, essentially 2 days with exposure and 2 days feeding elsewhere with no exposure.

Use of the highest value of PT obtained in this study, 0.66, can therefore serve as a conservative estimate for starlings as a focal species relevant for bare soil treated with Metam-sodium for carrot production.

KCP 10.1.1.2/03

[redacted] (2010). METAM SODIUM: A field study to determine residues in carrot field invertebrates as potential food items for birds and small mammals in SW France

MITOX Study Nr. T004IRW

This study was evaluated during EU review of the active substance metam, and considered acceptable.

However, Member States Experts at PRAPeR TC 54 pointed out several uncertainties regarding the representativeness of invertebrates and soil properties. Therefore, it was agreed to use the highest residue measured in the study (13.3 mg/kg) as RUD in the risk assessment for Birds and Mammals at EU level.

Since birds will need to consume a considerable quantity of arthropods to acquire a lethal dose and these will be distributed over a large surface area when compared to this study it is considered that the 90th centile residue represents a worst realistic residue case.

The highest residue measured in the study (13.3 mg/kg) was linked to the application rate of 612 kg as/ha Metam Na 510 SL (equivalent to 346 kg MITC/ha). When converting this highest measured residue value to a RUD taking into account the actual application rate of 612 kg as/ha Metam Na 510 SL this results in RUD values of 0.022 for Metam Na 510 SL and 0.038 for MITC. These adjusted maximum values are lower than the 90th percentile RUD of 0.146 used in the insectivorous birds risk assessment which was derived from the combination of the entire database of residues in arthropods so as to reduce uncertainty. Therefore, the use of the RUD of 0.146 covers the worst case situation.

Higher tier data on birds
KCP 10.1.1.2/04 [redacted] **(2009). Generic monitoring of birds in maize fields in France.** [redacted] This study was executed in maize fields. The pre-emergence maize fields can be considered to represent the same habitat as a bare field site treated with metam before planting. Consequently, data obtained for pre-emergence in maize in this study could be used in risk assessment for metam application under field conditions. As faecal samples were obtained from birds captured between early April and early June (pre-emergence), the PD values determined from these samples are also suitable for use in the risk assessment for metam application under field conditions. Scan samples confirmed the relevant focal species in maize pre and post emergence. This study provides measured estimates of PT and dietary composition for use in risk assessments for five species in maize fields. For Starling, a PD value of 0.76 was calculated (76 % animal matter found in faeces).
KCP 10.1.1.2/05 [redacted] **(2014a). A field study in arable land treated with metam-sodium 510 g/L to determine residues of MITC in field populations of earthworms as potential food items for birds and mammals.** **Syntech Study Nr. 201SRFR13C2** Maximum 90th percentile measured MITC residues Earthworms (digging and hand sorting): 137.6 mg MITC/kg (3 DAA of 612 kg a.s./ha / 346 kg MITC/ha) Earthworms (surface searching): 133.1 mg MITC/kg (1 DAA of 408 kg a.s./ha / 231 kg MITC/ha) Maximum measured MITC residues Earthworms (digging and hand sorting): 146.0 mg MITC/kg (3 DAA of 612 kg a.s./ha / 346 kg MITC/ha) Earthworms (surface searching): 156.0 mg MITC/kg (1 DAA of 408 kg a.s./ha / 231 kg MITC/ha)
KCP 10.1.1.2/06 [redacted] **(2014b). A field study in arable land treated with metam-sodium 510 g/L to determine residues of MITC in field populations of soil and surface dwelling arthropods as potential food items for birds and mammals. Field phase conducted in southern zone of Europe.** **Syntech Study Nr. 201SRFR13C3** Worst-case mean measured MITC residues Ground arthropods (pitfall traps): 72.51 mg MITC/kg (1 DAA at 306 kg metam/ha) Maximum measured MITC residues Ground arthropods (pitfall traps): 170 mg MITC/kg (at 306 kg metam/ha)
KCP 10.1.1.2/07 [redacted] **(2014). Metam, Higher Tier Avian Risk Assessment Body burden modelling approach for earthworm eating bird scenarios.** **Rifcon Report Nr. P1330323** The body burden modelling was performed according to the recommendations in the EFSA Guidance Document for Birds & Mammals (2009) and the PPR Opinion on pirimicarb (2005)[26], that describes the use of body burden modelling for another carbamate (pirimicarb) than metam. The proposed model can therefore be accepted. Based on data for a relevant indicator species (Mistle thrush), refined values for body weight absorption, distribution, metabolism, and excretion of metam in birds it is concluded that favourable-case assessment is a more realistic refinement of the exposure estimation. Hence, the acute risk to earthworm-eating birds resulting from applications of metam is considered acceptable.

[26] EFSA (2005). "Opinion of the scientific panel on plant health, plant protection products and their residues on a request from EFSA related to the evaluation of primicarb." The EFSA Journal **240**: 1-21.

Higher tier data on birds
KCP 10.1.1.2/08 **[redacted](2010). The Acute Risks to Birds and Mammals potentially exposed following proposed commercial use of Metam-sodium.** Glasshouse uses of metam-sodium are excluded from this risk assessment since there will be no exposure to mammals from closed crop systems. The field uses which distribute MITC widely throughout the soil, injection/rotavation, injection/coverage and drip irrigation are considered to present the greatest risk to mammals, since the majority of the soil dwelling arthropods and earthworms will receive almost uniform exposure. The toxicity of MITC to mammals has been assessed in acute tests. The value of 100 mg/kg bw/d (mouse) was suggested by EFSA.

Table 2.9.1.1-3: Summary of higher tier data submitted by the applicant Lainco

Higher tier data on birds
KCP 10.1.1.2/01 **[redacted] (2014). Determination of Residues of MITC (Degradation Product of Metam) in Arthropods, Earthworms, Soil and Weed Seeds after one Application of METAM SODIUM 51% SL in Soil at one Site in France in 2013.** The aim of this study was to determine residues levels of MITC (methylisothiocyanate), the degradation product of metam, in different food sources (arthropods, earthworms and weed seeds) for wild birds and mammals under field conditions following a single application of Metam Sodium 51% SL (batch number F2477, analysed content 51.96 % w/v). The residues in soil were also determined, both before and after application of the test item. This study was performed based on EFSA (2009)[27] and in accordance with GLP. The field site was located in Alsace, France. The study was conducted on bare soil (arable land). The field site design consisted of one test item treatment group including three replicates. The test item Metam Sodium 51% SL, with a content of 51.96 % w/v sodium methyldithiocarbamate (analysed), was applied by fumigation to the field site once in September 2013 at 300 L product/ha (153 kg metam sodium/ha, equivalent to 86.6 kg MITC/ha). Assessments of bird species, mice species and other mammals were conducted. The main species of birds present on the field were identified by visual observation. Assessments took place before the application (4 and 3 DBA) and at days 0, 1, 2 and 3 after the application. Mice present on the field were caught using baited live traps. The main species of large mammals present on the field were additionally identified through visual observations. Assessments took place before the application (4 and 3 DBA) and at days 0, 1, 2 and 3 after the application. The plant species present on the field site were identified by walking a transect across each plot. The assessment of plants in the field took place once before the application. Samples of potential bird/mammal food were collected for MITC residue analysis from the following sources: active epigeal arthropods (pitfall trap sampling), active and immobile epigeal arthropods (visual sampling), earthworms (hand sorting and visual sampling) and seeds (seed sampling). Soil samples for MITC analysis were taken in each plot from sampling points well distributed within the sampling zone. Soil cores were also taken for soil characterization. The determination of MITC residues was performed by HPLC with MS/MS detection (LOQ = 0.05 mg/kg for insect, earthworm and seed samples, LOQ = 0.01 mg/kg for soil samples). Environmental conditions were monitored during the study, including air temperature (min., max.), soil temperature (0-5 cm, 15-20 cm), soil moisture (0-5 cm, 15-20 cm), relative air humidity (min., max.) and precipitation using portable devices. The highest 90th percentile residue calculated for bird and mammal food items was for arthropods (pitfall traps), being 2.29 mg/kg. This was followed by anecic earthworms (1.90 mg/kg), other earthworms (1.64 mg/kg), earthworms (visual sorting, 0.75 mg/kg) and seeds (0.59 mg/kg). The maximum residues measured for bird and mammal food items were for arthropods (pitfall traps), being 2.66 mg/kg. This was followed by anecic earthworms (2.10 mg/kg), other earthworms (1.67 mg/kg), earthworms (visual sorting, 0.90 mg/kg) and seeds (0.59 mg/kg). The highest mean residues of MITC in soil samples were detected in the 5-10 cm and 10-15 cm horizons throughout the study period (0 DAA to 10 DAA). The maximum mean residue detected during the study was at 1 DAA, being 13.18 mg/kg at 5-10cm depth. The overall maximum residue detected during the study was at 0 DAA, being 14.95 at 5-10 cm depth.

[27] EFSA (2009) EFSA Guidance document on risk assessment for birds and mammals.

Higher tier data on birds

KCP 10.1.1.2/02

[redacted](2014a). A field study in arable land treated with metam sodium 510 g/L to determine residues of MITC in field populations of earthworms as potential food items for birds and mammals. Field phase conducted in southern zone of Europe.

This study was designed to determine residues of MITC (the metabolite of metam-sodium) in field populations of earthworms (Annelida: Oligochaeta) following one soil injection of the test item metam-sodium 510 g/L to arable land in southern zone of Europe. Samples of the natural populations of earthworms were collected and analysed for residues of MITC in order to estimate the potential dietary exposure of earthworm-feeding vertebrate consumers (farmland birds and mammals) to metam-sodium 510 g/L.

The study was conducted in east-central France, between Lyon (69000) and Chalon-sur-Saône (71100). There were five replicate field sites of approximately 1600 m² each. The sites were at least 15 km apart and situated on level ground with different soil types appropriate for growing some of the crops described in the GAP of the test item.

One to three weeks before the application all five field sites were mulched and then the soil was superficially ploughed (to a depth of 10 cm).

At each site 4 experimental plots were established, each 21 m x 21 m in size. One treatment was assigned to each plot. Treatments consisted of an untreated control, and the test item at three different application rates (306, 408 and 612 kg a.s./ha, corresponding to 600, 800 and 1200 L formulated product/ha respectively, based on the nominal a.s. content).

Samples of earthworms for residue analysis were collected by digging and hand sorting from a 1 m² area in the central area of each plot. Samples taken on the same date were at least 2 m apart and sampled areas were not used for sampling at subsequent sampling dates. Samples were taken before application then 1, 3 and 7 days after application. Additionally, soil surface searching for earthworms was conducted at days 0 (6-8 h after application) and 1 after application.

Concentrations of MITC in soil were determined once by soil core sampling 2-4 h after the application. Ten soil cores (2.5 cm diameter x 30 cm depth) were taken from each control and test item treatment plot. Samples were stored deep-frozen at ≤ -18 °C for transport to the analytical test site.

Specimens of earthworms and soil were stored frozen and shipped deep-frozen to the analytical test facility of Fera, United Kingdom for residue analysis.

At the lower rate of 306 kg a.s./ha, (equivalent to 173 kg/ha MITC) residues in earthworms sampled by digging one day after application ranged from 15.4 to 129 mg/kg. Three days after application residues in earthworms were between 29.9 mg/kg and 106 mg/kg. Seven days after application residues were between 6.1 and 85 mg/kg.

At the middle rate of 408 kg a.s./ha (equivalent to 231 kg/ha MITC) residues in earthworms sampled by digging on the day after application ranged from 17 to 95.5 mg/kg. Three days after application residues in earthworms were between 2.7 mg/kg and 91.8 mg/kg. Seven days after application residues were between 3.7 and 13.6 mg/kg.

At the higher rate of 612 kg a.s./ha, (equivalent to 346 kg/ha MITC) residues in earthworms sampled by digging one day after application ranged from 12 to 81.9 mg/kg. Three days after application residues in earthworms were between 1.2 mg/kg and 152 mg/kg. Seven days after application residues were between 30.7 and 136 mg/kg.

Two samples of earthworms from site 1 contained less than 0.1 g of material. These have been excluded as they skew the overall data and represent such a small fraction of a potential dietary intake.

Surface searching in plots treated at the lower rate of 306 kg a.s./ha, (equivalent to 173 kg/ha MITC) on the day of application collected earthworms with residues ranging from 0.85 to 57.3 mg/kg. Surface searching one day after application collected earthworms with residues of MITC between 36.3 mg/kg and 81.4 mg/kg.

Surface searching in plots treated at the middle rate of 408 kg a.s./ha, (equivalent to 231 kg/ha MITC) on the day of application collected earthworms with residues ranging from 13.6 to 91.3 mg/kg. Surface searching one day after application collected earthworms with residues of MITC between 12.1 mg/kg and 156 mg/kg.

Surface searching in plots treated at the higher rate of 612 kg a.s./ha, (equivalent to 346 kg/ha MITC) on the day of application collected earthworms with residues ranging from 11.9 to 112 mg/kg. Surface searching one day after application collected earthworms with residues of MITC between 71.3 mg/kg and 138 mg/kg.

Higher tier data on birds

KCP 10.1.1.2/03

[redacted] (2014b). A field study in arable land treated with metam sodium 510 g/L to determine residues of MITC in field populations of soil and surface dwelling arthropods as potential food items for birds and mammals. Field phase conducted in southern zone of Europe.

This study was designed to determine residues of MITC (the metabolite of metam-sodium) in field populations of soil and surface dweling arthropods following one soil injection of the test item metam-sodium 510 g/L to arable land in southern zone of Europe. Samples of the natural populations of arthropods were collected and analysed for residues of MITC in order to estimate the potential dietary exposure of invertebrate-feeding vertebrate consumers (farmland birds and mammals) to metam-sodium 510 g/L.

The study was conducted in South-East France, between Avignon (84000) and Montpellier (34000). There were five replicate field sites of approximately 1 ha each. The sites were at least 15 km apart and situated on level ground with different soil types appropriate for growing some of the crops described in the GAP of the test item.

At each site 4 experimental plots were established, each 51 m x 51 m in size. One treatment was assigned to each plot. Treatments consisted of an untreated control, and the test item at three different application rates (306, 408 and 612 kg a.s./ha, corresponding to 600, 800 and 1200 L formulated product/ha respectively, based on the nominal a.s. content).

Samples of arthropods for residue analysis were collected using dry pitfall traps. Samples were taken before application (48-hour sampling period immediately before application) then during sampling periods at 0-6 h, 6-12 h, 12-24 h, 24-48 h and 48-96 h after application. Additional soil surface searching were conducted at days 0 (6-8 h after application) and 1 after application. Collected arthropods were placed into the four aluminium foil-lined plastic containers corresponding to their respective group (adult beetles, larvae, spiders, others), then immediately weighed and stored deep frozen at ≤ -18 °C for transport to the analytical test site of Fera, United Kingdom for residue analysis.

Concentrations of MITC in soil were determined once by soil core sampling 2-4 h after the application. Ten soil cores (2.5 cm diameter x 30 cm depth) were taken from each control and test item treatment plot. Samples were stored deep-frozen at ≤ -18 °C for transport to the analytical test site.

Specimens of earthworms and soil were stored frozen and shipped deep-frozen to the analytical test facility of Fera, United Kingdom for residue analysis.

All pre-treatment samples contained residues of MITC of <0.05 mg/kg.

Samples from untreated control plots contained residues of MITC ranging from <0.05 to 3.99 mg MITC/kg, presumed to be as a result of arthropods moving from one experimental plot to another. At the five sites the maximum MITC residue in arthropods was 2.13 (larvae at Site 1), 3.99 (spiders at Site 2), 2.04 (other arthropods at Site 3), 2.0 (spiders at Site 4) and 0.46 mg MITC/kg (other arthropods at Site 5), respectively.

At the lower rate of 306 kg a.s./ha, (equivalent to 173 kg/ha MITC) residues in arthropods sampled using pitfall traps ranged from <0.05 to 170 mg MITC/kg. Of the 60 samples analysed 27 contained MITC residues of <0.05 mg MITC/kg.

At the middle rate of 408 kg a.s./ha (equivalent to 231 kg/ha MITC) residues in arthropods sampled using pitfall traps ranged from <0.05 to 92.4 mg MITC/kg. Of the 51 samples analysed 20 contained MITC residues of <0.05 mg MITC/kg.

At the higher rate of 612 kg a.s./ha, (equivalent to 346 kg/ha MITC) residues in arthropods sampled using pitfall traps ranged from <0.05 to 113 mg MITC/kg. Of the 53 samples analysed 23 contained MITC residues of <0.05 mg MITC/kg.

Surface searching in all treated plots only yielded 20 samples for analysis with residues ranging from <0.05 mg MITC/kg to 124 mg MITC/kg. Of the 19 samples analysed 10 contained MITC residues of <0.05 mg MITC/kg.

Across all three treatment rates there was no clear trend of higher or lower residues being found in any one of the four arthropod categories analysed (beetles, spiders, larvae and others).

2.9.1.2 Mammals

Mammalian toxicity studies were conducted with the rat for either metam-sodium or the active metabolite MITC. A summary of the toxicity endpoints in shown in Table 2.9.1.2-1 and Table 2.9.1.2-2.

Table 2.9.1.2-1: Summary of acute mammalian endpoints for the active substance metam and its metabolite

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	Metam-sodium	Single dose (Acute oral toxicity)	Oral LD_{50}	896 mg a.s./kg bw	[redacted] 1991
Rat	MITC	Single dose (Acute oral toxicity)	Oral LD_{50}	147 mg/kg bw	[redacted], 1986a
Rat	MITC	Single dose (Acute oral toxicity)	Oral LD_{50}	**100 mg/kg bw**	EFSA Journal 2011; 9(9):2334*
Rat	MITC	Single dose (Acute whole-body inhalation toxicity)	Inhalation LC_{50}	**0.54 mg/L (air) (4 hour)**	[redacted], 1981

Note: ***bold*** *– endpoint used for the current risk assessment*

** for the ecotoxicological risk assessment the worst-case acute toxicity endpoint for MITC (from the dazomet dossier) LD_{50} (female mouse) = 100 mg MITC/kg bw has been used*

Table 2.9.1.2-2: Summary of short-term and long-term mammalian endpoints for the active substance metam and its metabolite

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	Metam-sodium	2-year oral (drinking water) toxicity and carcinogeniticy study	NOAEL	1.5 mg a.s./kg bw/day	[redacted], 1991 [redacted], 1994
Rat	MITC	28 day (Subchronic inhalation toxicity)	NOAEL	**5 mg/m³ (air)** (1.35 mg/kg bw/day)	[redacted], 1987
Rat	MITC	2-year oral (drinking water) toxicity and carcinogenicity study	NOAEL	**0.44 mg/kg bw/day**	[redacted], 1984

Note: ***bold*** *– endpoint used for the current risk assessment*

Higher tier data intended to refine exposure estimates (such as residues in bird and mammal food items, focal species, body burden modelling investigation) for outdoor uses of metam were submitted. Reference is made to Table 2.9.1.1-2 and Table 2.9.1.1-3.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

Aquatic toxicity studies were conducted with fish, aquatic invertebrates, algae and aquatic plants for either metam, the active metabolite MITC or the impurity DMTU. A summary of the toxicity endpoints is shown in Table 2.9.2-1.

Table 2.9.2-1: Summary of aquatic toxicity data on metam, the active metabolite MITC and the impurity DMTU

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Fish					
Rainbow trout (*Oncorhynchus mykiss*)	Metam-potassium (PNMDC)	96 hours, acute (flow-through)	LC_{50}	62.4 mg a.s./L[M]	CA8.2.1/01 [redacted], 1992 (not submitted by Lainco)
Rainbow trout (*Oncorhynchus mykiss*)	Vapam (32.7 % metam-sodium)	96 hours, acute (static)	LC_{50}	0.24 mg Vapam/L[N] (0.078 mg a.s./L)[N]	CA8.2.1/02 [redacted], 1971
Bluegill sunfish (*Lepomis macrochirus*)	Metam-sodium	96 hours, acute (static)	LC_{50}	> 0.464 mg a.s./L[N] > 0.175 mg a.s./L[M at 96 h] > 0.522 mg a.s./L[M*]	CA8.2.1/03 [redacted] 1986 CA8.2.1/04 [redacted], 2019a
Bluegill sunfish (*Lepomis macrochirus*)	Metam-potassium (PNMDC)	96 hours, acute (flow-through)	LC_{50}	108 mg a.s./L[M]	CA8.2.1/05 [redacted], 1992 (not submitted by Lainco)
Bluegill sunfish (*Lepomis macrochirus*)	Vapam (32.7 % metam-sodium)	96 hours, acute (static)	LC_{50}	1.19 mg Vapam/L[N] (0.389 mg a.s./L)[N]	CA8.2.1/06 [redacted], 1970a
Sheepshead minnow (*Cyprinodon variegatus*)	Metam-potassium (PNMDC)	96 hours, acute (flow-through)	LC_{50}	30 mg a.s./L[M]	CA8.2.1/07 [redacted], 1992 (not submitted by Lainco)
Sheepshead minnow (*Cyprinodon variegatus*)	Vapam (32.7 % metam-sodium)	96 hours, acute (static)	LC_{50}	1.3 mg Vapam/L[N] (0.425 mg a.s./L)[N]	CA8.2.1/08 [redacted], 1970b
Striped majatis	Vapam (32.7 % metam-sodium)	96 hours, acute (static)	LC_{50}	1.5 mg Vapam/L[N] (0.491 mg a.s./L)[N]	CA8.2.1/09 [redacted] 1970c
Rainbow trout (*Oncorhynchus mykiss*)	MITC	96 hours, acute (semi-static)	LC_{50}	**0.0531 mg/L[M (u)]**	CA8.2.1/10 [redacted], 2002
Rainbow trout (*Oncorhynchus mykiss*)	MITC	96 hours, acute (flow-through)	LC_{50}	0.094 mg/L [M (u)]	CA8.2.1/11 [redacted], 1991a

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Bluegill sunfish (*Lepomis macrochirus*)	MITC	96 hours, acute (flow-through)	LC_{50}	0.142 mg/L[M (u)]	CA8.2.1/12 [redacted], 1991b
Sheepshead minnow (*Cyprinodon variegatus*)	MITC	96 hours, acute (flow-through)	LC_{50}	115 µg/L[M]	CA8.2.1/13 [redacted], 2012a (not submitted by Lainco)
Rainbow trout (*Oncorhynchus mykiss*)	MITC	96 hours, acute (flow-through)	LC_{50}	90 µg/L[M]	CA8.2.1/14 [redacted] 2019a (not submitted by Lainco)
Rainbow trout (*Oncorhynchus mykiss*)	DMTU	96 hours, acute (semi-static)	LC_{50}	> 96.0 mg/L[M]	CA8.2.1/15 [redacted], 2011a
Rainbow trout (*Oncorhynchus mykiss*)	MITC	28 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	0.005 mg/L[N] 0.004 mg/L[MM] 0.022 mg/L[N] 0.017 mg/L[N]	CA8.2.1/01 [redacted], 1990 CA8.2.2/02 [redacted], 2019b (not submitted by Lainco)
Fathead minnow (*Pimephales promelas*)	MITC	33 days, chronic (ELS) (flow-through)	NOEC EC_{20} EC_{10}	7.74 µg/L[M] 11.3 µg/L[M] **9.24 µg/L[M]**	CA8.2.2.1/01 [redacted], 2015
Aquatic invertebrates					
Water flea (*Daphnia magna*)	Metam Fluid 510 g/L (42.2 % metam-sodium)	48 hours, acute (static)	EC_{50}	2.34 mg Metam Fluid/L[N] (0.99 mg a.s./L)[N]	CA8.2.4.1/01 [redacted], 1985
Water flea (*Daphnia magna*)	Metam-potassium (PNMDC)	48 hours, acute (flow-through)	EC_{50}	6.34 mg a.s./L[M]	CA8.2.4.1/02 [redacted]., 1993 (not submitted by Lainco)
Water flea (*Daphnia magna*)	Metam-sodium	48 hours, acute (semi-static)	EC_{50}	0.166 mg a.s./L[M]	CA8.2.4.1/03 [redacted], 2013
Water flea (*Daphnia magna*)	MITC	48 hours, acute (semi-static)	EC_{50}	**0.076 mg/L[M]**	CA8.2.4.1/04 [redacted]., 2002
Water flea (*Daphnia magna*)	MITC	48 hours, acute (flow-through)	EC_{50}	0.124 mg/L[M]	CA8.2.4.1/06 [redacted], 2019a
Water flea (*Daphnia magna*)	DMTU	48 hours, acute (static)	EC_{50}	30.4 mg/L[N]	CA8.2.4.1/07 [redacted]., 2013 (not submitted by Lainco)
Water flea (*Daphnia magna*)	DMTU	48 hours, acute (static)	EC_{50}	24.8 mg/L[M]	CA8.2.4.1/08 [redacted], 2011b

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Saltwater mysid (*Americamysis bahia*)	Metam-potassium (PNMDC)	96 hours, acute (flow-through)	EC_{50}	2.19 mg a.s./L[M]	CA8.2.4.2/01 [redacted], 1992 (not submitted by Lainco)
Eastern oyster (*Crassostrea virginica*)	Metam-potassium (PNMDC)	96 hours, acute (flow-through)	EC_{50} (growth)	6.45 mg a.s./L[M]	CA8.2.4.2/02 [redacted], 1993 (not submitted by Lainco)
Midge (*Chironomus riparius*)	MITC	48 hours, acute (semi-static)	EC_{50}	55 µg/L[M]	CA8.2.4.2/03 [redacted], 2018a
Midge (*Chironomus riparius*)	MITC	48 hours, acute (static)	EC_{50}	0.36 mg/L[M]	CA8.2.4.2/04 [redacted] 2014c (not submitted by Lainco)
Midge (*Chironomus riparius*)	MITC	48 hours, acute (semi-static)	EC_{50}	90.6 µg/L[M]	CA8.2.4.2/05 [redacted], 2014a
Freshwater amphipod (*Hyalella azteca*)	MITC	48 hours, acute (static)	LC_{50}	3.8 µg/L[M]	CA8.2.4.2/06 [redacted], 2014a (not submitted by Lainco)
Marine amphipod (*Leptocheirus plumulosus*)	MITC	48 hours, acute (static)	LC_{50}	0.16 mg/L[M]	CA8.2.4.2/07 [redacted], 2014b (not submitted by Lainco)
Saltwater midge (*Americamysis bahia*)	MITC	96 hours, acute (flow-through)	EC_{50}	55 µg/L[M]	CA8.2.4.2/08 [redacted], 2011 (not submitted by Lainco)
Eastern oyster (*Crassostrea virginica*)	MITC	96 hours, acute (flow-through)	EC_{50}	42 µg/L[M]	CA8.2.4.2/09 [redacted], 2012b (not submitted by Lainco)
Crustacean (*Asellus aquaticus*)	MITC	96 hours, acute (semi-static)	EC_{50}	0.11 mg/L[M]	CA8.2.4.2/10 [redacted], 2015 (not submitted by Lainco)
Crustacean (*Thamnocephalus platyurus*)	MITC	24 hours, acute (static)	LC_{50}	2.0 mg/L[N]	CA8.2.4.2/11 [redacted], 2014a (not submitted by Lainco)
Rotifers (*Brachionus calyciflorus*)	MITC	24 hours, acute (static)	LC_{50}	> 1.2 mg/L[M]	CA8.2.4.2/12 [redacted], 2014b (not submitted by Lainco)

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Freshwater oligochaete (*Lumbriculus variegatus*)	MITC	96 hours, acute (semi-static)	LC_{50}	0.315 mg/L[M]	CA8.2.4.2/13 [redacted], 2019a
Freshwater mudsnail (*Potamopyrgus antipodarum*)	MITC	96 hours, acute (semi-static)	LC_{50}	0.319 mg/L[M]	CA8.2.4.2/14 [redacted] 2019b
Brown flatworm (*Dugesia tigrina*)	MITC	96 hours, acute (semi-static)	LC_{50}	0.137 mg/L[N]	CA8.2.4.2/15 [redacted], 2019c
Freshwater crustacean amphipod (*Crangonyx pseudogracilis*)	MITC	48 hours, acute (semi-static)	LC_{50}	0.312 mg/L[M]	CA8.2.4.2/16 [redacted]., 2014b
Freshwater oligochaete (*Lumbriculus variegatus*)	MITC	48 hours, acute (semi-static)	LC_{50}	0.205 mg/L[M]	CA8.2.4.2/17 [redacted], 2014c
Water flea (*Daphnia magna*)	MITC	21 days, chronic (semi-static)	NOEC EC_{20} EC_{10}	0.00625 mg/L[N (u)] 9.970 µg/L[N (u)] 7.218 µg/L[N (u)]	CA8.2.5.1/01 [redacted], 2001 CA8.2.5.1/02 [redacted], 2019c Due to the uncertainty, the endpoints are not appropriate for risk assessment, preference is given to endpoints from study CA8.2.5.1/03
Water flea (*Daphnia magna*)	MITC	21 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	21.1 µg/L[M] 42 µg/L[M] **35 µg/L[M]**	CA8.2.5.1/03 [redacted]., 2019c
Algae					
Green microalgae (*Pseudo-kirchneriella subcapitata*)	Metam Sodium 510 g/L	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 96h NOEC	1.69 mg form/L[I] (0.556 mg a.s./L)[I] 0.075 mg a.s./L[I] ND ND ND ND 3.08 mg form/L[I] (1.08 mg a.s./L)[I] ND ND 1 mg form/L[I] (0.322 mg a.s./L)[I]	CA8.2.6.1/01 [redacted] 2003 CA8.2.6.1/02 [redacted], 2020a

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Green microalgae (*Pseudo-kirchneriella subcapitata*)	Metam Sodium (technical grade)	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h $NOEC_y$ 72h $NOEC_b$ 72h $NOEC_r$	0.117 mg a.s./L[M] 0.057 mg a.s./L[I] 0.0482 mg a.s./L[M] 0.118 mg a.s./L[M] 0.076 mg a.s./L[I] 0.061 mg a.s./L[I] 0.339 mg a.s./L[M] 0.134 mg a.s./L[I] 0.0779 mg a.s./L[M] 0.0378 mg a.s./L[M] 0.0378 mg a.s./L[M] 0.0813 mg a.s./L[M]	CA8.2.6.1/03 [redacted]., 2011 CA8.2.6.1/04 [redacted] 2020b
Green microalgae (*Pseudo-kirchneriella subcapitata*)	MITC	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	0.281 mg/L[I] ND ND ND ND ND 0.432 mg/L[I] ND ND ND	CA8.2.6.1/05 [redacted]1998 CA8.2.6.1/06 [redacted] 2019a (not submitted by Lainco)
Green microalgae (*Pseudo-kirchneriella subcapitata*)	MITC	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h $NOEC_y$ 72h $NOEC_r$	ND 0.10 mg/L[I] 0.08 mg/L[I] 0.12 mg/L[I] 0.09 mg/L[I] 0.08 mg/L[I] 0.21 mg/L[I] 0.13 mg/L[I] 0.09 mg/L[I] 0.044 mg/L[I] 0.11 mg/L[I]	CA8.2.6.1/07 [redacted]., 2012d CA8.2.6.1/08 [redacted] 2020c (not submitted by Lainco)
Green microalgae (*Pseudo-kirchneriella subcapitata*)	MITC	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	93.3 µg/L[M] 58.3 µg/L[M] 45.6 µg/L[M] 91 µg/L[M] 62 µg/L[M] 51 µg/L[M] **189 µg/L[M]** 104 µg/L[M] 76 µg/L[M] ND	CA8.2.6.1/09 [redacted]., 2018b CA8.2.6.1/10 [redacted] 2020d

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Green microalgae (*Pseudo-kirchneriella subcapitata*)	DMTU	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	ND ND ND > 104 mg/L[M] ND ND > 104 mg/L[M] ND ND ND	CA8.2.6.1/11 [redacted]., 2011c (not submitted by Eastman (Taminco))
Blue-green Alga (*Anabaena flos-aquae*)	MITC	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	1.886 mg/L[I] ND 0.793 mg/L[I] 2.12 mg/L[I] ND 0.65 mg/L[I] 3.607 mg/L[I] ND 1.143 mg/L[I] ND	CA8.2.6.2/01 [redacted], 2002 CA8.2.6.2/02 [redacted] [redacted], 2019b (not submitted by Lainco)
Blue-green Alga (*Anabaena flos-aquae*)	MITC	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	179.2 µg/L[I] 89.0 µg/L[I] 61.7 µg/L[I] 0.431 mg/L[I] ND ND 0.433 mg/L[I] ND ND ND	CA8.2.6.2/03 [redacted] [redacted]., 2012a CA8.2.6.2/04 [redacted] [redacted], 2020 (not submitted by Lainco)
Freshwater diatom (*Navicula pelliculosa*)	MITC	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	180.4 µg/L[I] 116.0 µg/L[I] 92.1 µg/L[I] 0.181 mg/L[I] ND ND 0.349 mg/L[I] 196.6 µg/L[I] 144.0 µg/L[I] ND	CA8.2.6.2/05 [redacted] [redacted], 2012b CA8.2.6.2/06 [redacted] [redacted] 2020a (not submitted by Lainco)

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Marine diatom (*Skeletonema costatum*)	MITC	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	139.1 µg/L[I] 40.2 µg/L[I] 21.0 µg/L[I] 0.081 mg/L[I] 37.2 µg/L[I] 24.6 µg/L[I] > 0.430 mg/L[I] ND 35.1 µg/L[I] ND	CA8.2.6.2/07 [redacted]., 2012c CA8.2.6.2/08 [redacted]., 2020b (not submitted by Lainco)
Blue-green Alga (*Anabaena flos-aquae*)	MITC	72 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} 72h NOEC	208 µg/L[N] 99 µg/L[N] 67 µg/L[N] 0.181 mg/L[N] 0.079 mg/L[N] 0.051 mg/L[N] 0.375 mg/L[N] 0.226 mg/L[N] 0.173 mg/L[N] ND	CA8.2.6.2/09 [redacted], 2019 CA8.2.6.2/10 [redacted]., 2020e
Aquatic plants					
Lemna gibba	MITC	7 days, chronic (semi-static)	Frond number E_bC_{50} E_bC_{20} E_bC_{10} E_yC_{50} E_yC_{20} E_yC_{10} E_rC_{50} E_rC_{20} E_rC_{10} NOEC	 0.556 mg/L[M (u)] ND 0.160 mg/L[M (u)] ND ND ND 1.133 mg/L[M (u)] ND 0.411 mg/L[M (u)] ND	CA8.2.7/01 [redacted], 2002 CA8.2.7/02 [redacted]., 2019d (not submitted by Lainco)
Lemna gibba	MITC	7 days, chronic (flow-through)	Frond number E_bC_{50} E_bC_{20} E_bC_{10} E_yC_{50} E_yC_{20} E_yC_{10} E_rC_{50} E_rC_{20} E_rC_{10} NOEC	 ND ND ND ND ND ND **0.29 mg/L[M]** 0.17 mg/L[M] 0.13 mg/L[M] ND	CA8.2.7/03 [redacted], 2019c (not submitted by Eastman (Taminco))

Notes: ***bold*** *– values used for risk assessment*

[M] *– based on mean measured concentrations unless otherwise stated*

N	*– based on nominal concentration(s)*
I	*– based on initial measured concentration(s)*
ND	*– could not be determined*
NE	*– not estimated*
*	*- statistical re-analysis*
(u)	*- uncertainty on the endpoint determination due to analytical method validation*

Further information on the available studies and a discussion of the results in relation to the CLP classification is provided in the sections below.

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

The estimation of bioaccumulation potential in fish is based on the partition coefficient n-octanol/water (log P_{OW}) of the active substance. In the section on physico-chemical properties different values for the log P_{OW} pending on the pH were measured.
The log P_{OW} values are then compared with the threshold values for bioaccumulation (threshold CLP ≥ 4). The log P_{OW} of metam is < -2.0, and the log P_{OW} of MITC is 1.05. Both values are lower than the threshold. Therefore, no experimental bioaccumulation data are required. Metam and MITC show a low potential for bioaccumulation

Table 2.9.2.1-1 Summary of relevant information on bioaccumulation for metam and MITC

Method	Species	Results	Key or Supportive study	Remarks	Reference
Metam					
EEC A.8 OECD 107 (Shake-flask method) GLP (Partition coefficient n-octanol/water)	-	log P_{ow} = < -2.0 (rounded from – 2.32, pH 7, 21.2°C) log P_{ow} = < -2.0 (rounded from – 2.39, pH 9, 21.2°C)	Key study	Metam-sodium Hydrate, 101.84%	[redacted]. (2020) Report No. CQ47NS KCA 2.7/02 (Taminco)
MITC					
EEC A8 (Shake-flask method) GLP (Partition coefficient n-octanol/water)	-	log P_{ow} at 20°C: 1.05 (pH 7.5)	Key study	-	[redacted](1997) Report No. FCC 153/962827 KCA 2.7/04

2.9.2.1.1 Estimated bioaccumulation

No data available and not required (see 2.9.2.1).

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

A study measuring the log P_{OW} is available for both metam and MITC. The results of these studies are summarized in Table 2.9.2.1-1. The measured log P_{OW} is < -2.0 for metam and 1.05 for MITC.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

The relevant studies on the acute aquatic toxicity of metam are shown in Table 2.9.2.2-1 below. The relevant studies on the acute aquatic toxicity of MITC are shown in Table 2.9.2.2-2. These table contain all information currently available in the dossier submitted for the current Annex I renewal application of metam.

Table 2.9.2.2-1 Summary of relevant information on acute aquatic toxicity for metam

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
Fish						
Acute fish study based on US EPA 72-1 GLP	*Oncorrhynchus mykiss*	Metam-potassium, purity: 54.0%, Batch no.: 1A-1275	LC_{50} = 62.4 mg a.s./L (mean measured)	Acceptable. Supportive study	96 h flow-through 20 fish/replicate 1 replicate/ treatment	CA8.2.1/01 [redacted], 1992
Acute fish study, No specific guideline followed Not GLP	*Oncorrhynchus mykiss*	Metam-sodium, Purity: 32.7%, Batch no.: not reported	LC_{50} = 0.078 mg a.s./L (nominal)	Not acceptable for classification	96 h static 10 fish/replicate 2 replicates/ treatment No analytical measurements, no detailed information on test material and test conditions	CA8.2.1/02 [redacted], 1971
Acute fish study based on US EPA 72-1 Not GLP	*Lepomis macrochirus*	Metam-sodium, Purity: 42.2%, Batch no.: ZH 130 585	LC_{50} > 0.522 mg a.s./L (mean measured)	Acceptable. Key study	96 h static 10 fish/treatment 1 replicate/ treatment	CA8.2.1/03 [redacted], 1986 CA8.2.1/04 [redacted], 2019 a
Acute fish study based on US EPA 72-1 GLP	*Lepomis macrochirus*	Metam-potassium, Purity: 54.0%, Batch No.: 1A-1275	LC_{50} = 108 mg a.s./L (mean measured)	Acceptable Supportive study	96 h flow-through 10 fish/replicate 2 replicates/ treatment	CA8.2.1/05 [redacted]., 1992
Acute fish study, No specific guideline followed Not GLP	*Lepomis macrochirus*	Metam-sodium, Purity: 32.7%, Batch no.: not reported	LC_{50} = 0.389 mg a.s./L (nominal)	Not acceptable for classification	96 h static 5 fish/replicate 4-2 replicates/ treatment No analytical measurements, no detailed information on test material and test conditions	CA8.2.1/06 [redacted]., 1970a
Acute fish study based on US EPA 72-3	*Cyprinodon variegatus*	Metam-potassium, Purity: 54.0%,	LC_{50} = 30 mg a.s./L (mean measured)	Acceptable Supportive study	96 h flow-through 20 fish/replicate	CA8.2.1/07 [redacted],

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
GLP		Batch No.: 1A-1275			1 replicate/ treatment	1992
Acute fish study, No specific guideline followed Not GLP	*Cyprinodon variegatus*	Metam-sodium, Purity: 32.7%, Batch no.: not reported	LC_{50} = 0.425 mg a.s./L (nominal)	Not acceptable for classification	96 h static 5 fish/replicate 4-2 replicates/ treatment No analytical measurements, no detailed information on test material and test conditions	CA8.2.1/08 [redacted], 1970b
Acute fish study, No specific guideline followed Not GLP	Striped majatis	Metam-sodium, Purity: 32.7%, Batch no.: not reported	LC_{50} = 0.491 mg a.s./L (nominal)	Not acceptable for classification	96 h static 5 fish/replicate 4-2 replicates/ treatment No analytical measurements, no detailed information on test material and test conditions	CA8.2.1/09 [redacted], 1970c
Aquatic invertebrates						
Acute daphnia study based on US EPA 72-2 not GLP	*Daphnia magna*	Metam-sodium, Purity: 42.2 %, Batch no.: not reported	EC_{50} = 0.99 mg a.s./L (nominal)	Not acceptable for classification	48 h static 5 daphnids/ replicate 4 replicates/ treatment No analytical measurements, no detailed information on test material	CA8.2.4.1/01 [redacted] 1985
Acute daphnia study based on US EPA 72-2 GLP	*Daphnia magna*	Metam-potassium, Purity: 54.0 %, Batch no.: 1A-1275	EC_{50} = 6.34 mg a.s./L (mean measured)	Acceptable Supportive study	48 h flow-through 10 daphnids/ replicate 2 replicates/ Treatment	CA8.2.4.1/02 [redacted]., 1993
Acute daphnia study based on EC 440/2008 Part C Method2, OECD 202 GLP	*Daphnia magna*	Metam-sodium, Purity: 51.99 %, Batch no.: E4227	EC_{50} = 0.166 mg a.s./L (mean measured)	Acceptable Key study	48 h semi-static 5 daphnids/ replicate, 4 replicates/ Treatment	CA8.2.4.1/03 [redacted], 2013
Acute invertebrate study based on US EPA 72-3	*Americamysis bahia*	Metam-potassium, Purity: 54.0 %, Batch no.: 1A-	EC_{50} = 2.19 mg a.s./L (mean measured)	Acceptable Supportive study	96 h flow-through 10 mysids/ replicate 2 replicates/	CA8.2.4.2/01 [redacted], 1992

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
GLP		1275			treatment	
Acute invertebrate study based on US EPA 72-3 GLP	*Crassostrea virginica*	Metam-potassium, Purity: 54.0 %, Batch no.: 1A-1275	EC_{50} = 6.45 mg a.s./L (mean measured)	Acceptable Supportive study	96 h flow-through 20 oysters/ replicate 1 replicate/ treatment	CA8.2.4.2/02 [redacted] [redacted] [redacted]., 1993
Algae						
Algal growth inhibtion study based on OECD 201, EU 92/69/EEC Part C2, JMAFF, US EPA OPPTS 850.5400 GLP	*Pseudokirchne-riella subcapitata*	Metam-sodium 510 g/L, Batch no.: 32 E 28/6	E_bC_{50} (72 h) = 0.556 mg a.s./L E_rC_{50} (72 h) = 1.08 mg a.s./L NOEC (96 h) = 0.322 mg a.s./L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 1 x 10^4 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/01 [redacted], 2003
Algal growth inhibition study based on EC 92/69/EEC Part C Method 3, OECD 201 GLP	*Pseudokirchne-riella subcapitata*	Metam-sodium, Purity: 44.7 %, Batch no.: B3698	E_yC_{50} = 0.118 mg a.s./L E_yC_{10} = 0.061 mg a.s./L E_bC_{50} = 0.117 mg a.s./L E_bC_{10} = 0.0482 mg a.s./L E_rC_{50} = 0.339 mg a.s./L E_rC_{10} = 0.0779 mg a.s./L $NOEC_y$ = $NOEC_b$ = 0.0378 mg a.s./L $NOEC_r$ = 0.0813 mg a.s./L (mean measured)	Acceptable Key study	72 h static Initial cell count: 1 x 10^4 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/03 [redacted]., 2011

Table 2.9.2.2-2 Summary of relevant information on acute aquatic toxicity for MITC

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
Fish						
Acute fish study based on US EPA 72-1 and OECD 203 GLP	*Oncorrhynchus mykiss*	MITC, Purity: 99.6%, Batch no.: 408208/1	LC_{50} = 0.0531 mg MITC/L (mean measured)	Acceptable Key study	96 h semi-static 7 fish/replicate 1 replicate/ treatment	CA8.2.1/10 [redacted] 2002
Acute fish study based on US EPA 72-1 Not GLP	*Oncorrhynchus mykiss*	MITC, Purity: 94.9%, Batch no.: not reported	LC_{50} = 0.094 mg MITC/L (mean measured)	Acceptable Supportive study	96 h flow-through 10 fish/replicate 2 replicates/ treatment	CA8.2.1/11 [redacted]., 1991a
Acute fish study based on US EPA 72-1 Not GLP	*Lepomis macrochirus*	MITC, Purity: 94.9%, Batch no.: not reported	LC_{50} = 0.142 mg MITC/L (mean measured)	Acceptable Supportive study	96 h flow-through 10 fish/replicate 2 replicates/ treatment	CA8.2.1/12 [redacted]., 1991b
Acute fish study based on US EPA OPPTS Nb. 850.1075 GLP	*Cyprinodon variegatus*	MITC, Purity: 99.7%, Batch no.: 56198PJV	LC_{50} = 0.115 mg MITC/L (mean measured)	Acceptable Supportive study	96 h flow-through 10 fish/replicate 2 replicates/ treatment	CA8.2.1/13 [redacted], 2012a
Acute fish study based on OECD 203 GLP	*Oncorrhynchus mykiss*	MITC, Purity: 99.6%, Batch no.: STBB1308V	LC_{50} = 0.090 mg MITC/L (mean measured)	Acceptable Supportive study	96 h flow-through 7 fish/replicate 1 replicate/ treatment	CA8.2.1/14 [redacted], 2019a
Aquatic invertebrates						
Acute daphnia study based on OECD 202, EEC 79/831 Annex V Part C2, US EPA 72-2, OPPTS 850.1010 GLP	*Daphnia magna*	MITC, Purity: 99.6 %, Batch no.: 48208/1	EC_{50} = 0.076 mg MITC/L (mean measured)	Acceptable Supportive study	48 h semi-static 5 daphnids/ replicate, 4 replicates/ treatment	CA8.2.4.1/04 [redacted]., 2002
Acute daphnia study based on OECD 202, EC 440/2008 Method C.2 GLP	*Daphnia magna*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	EC_{50} = 0.124 mg MITC/L (mean measured)	Acceptable Supportive study	48 h flow-through 5 daphnids/ replicate 4 replicates/ treatment	CA8.2.4.1/06 [redacted], 2019a
Acute	*Chironomus*	MITC, Purity:	EC_{50} =	Acceptable	48 h semi-	CA8.2.4.2/0

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
invertebrate study based on OECD 235 GLP	*riparius*	99.6 %, Batch no.: STBB1308V	0.055 mg MITC/L (mean measured)	Supportive study	static 5 midges/ replicate 4 replicates/ treatment	3 [redacted], 2018a
Acute invertebrate study based on OECD 235 GLP	*Chironomus riparius*	MITC, Purity: 97.2 %, Batch no.: 56198PJV	EC_{50} = 0.36 mg MITC/L (mean measured)	Acceptable Supportive study	48 h static 5 midges/ replicate 4 replicates/ treatment	CA8.2.4.2/04 [redacted], 2014c
Acute invertebrate study based on OECD 235 GLP	*Chironomus riparius*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	EC_{50} = 0.0906 mg MITC/L (mean measured)	Acceptable Supportive study	48 h semi-static 5 midges/ replicate 4 replicates/ treatment	CA8.2.4.2/05 [redacted], 2014a
Acute invertebrate study based on OECD 202, US EPA OPPTS 850.1010 GLP	*Hyalella azteca*	MITC, Purity: 97.2 %, Batch no.: 56198PJV	LC_{50} = 0.0038 mg MITC/L (mean measured)	Acceptable Key study	48 h static 5 amphipods/ replicate 4 replicates/ treatment	CA8.2.4.2/06 [redacted], 2014a
Acute invertebrate study based on OECD 202, US EPA OPPTS 850.1010 GLP	*Leptocheirus plumulosus*	MITC, Purity: 97.0 %, Batch no.: 51698PJV	LC_{50} = 0.16 mg MITC/L (mean measured)	Acceptable Supportive study	48 h static 5 amphipods/ replicate 4 replicates/ treatment	CA8.2.4.2/07 [redacted], 2014b
Acute invertebrate study based on US EPA OPPTS 850.1035 GLP	*Americamysis bahia*	MITC, Purity: 99.7 %, Batch no.: 51698PJV	LC_{50} = 0.055 mg MITC/L (mean measured)	Acceptable Supportive study	96 h flow-through 10 mysids/ replicate 2 replicates/ treatment	CA8.2.4.2/08 [redacted], 2011
Acute invertebrate study based on US EPA OPPTS 850.1025 GLP	*Crassostrea virginica*	MITC, Purity: 98.9 %, Batch no.:56198PJV	EC_{50} = 0.042 mg MITC/L (mean measured) Endpoint based on growth	Acceptable Supportive study	96 h flow-through 20 oysters/ replicate 1 replicate/ treatment	CA8.2.4.2/09 [redacted], 2012b
Acute invertebrate study based on US EPA 850.1020 GLP	*Asellus aquaticus*	MITC, Purity: 98.9 %, Batch no.: A0337662	LC_{50} = 0.11 mg MITC/L (mean measured)	Acceptable Supportive study	96 h semi-static 20 organisms/ replicate 1 replicate/	CA8.2.4.2/10 [redacted], 2015

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
					treatment	
Acute invertebrate study based on OECD 202, OPPTS 850.1010 GLP	*Thamnocephalus platyurus*	MITC, Purity: 98.9 %, Batch no.: A0337662	LC_{50} = 2.0 mg MITC/L (nominal)	Acceptable Supportive study	24 h static 10 organisms/ replicate 3 replicates/ treatment	CA8.2.4.2/11 [redacted], 2014a
Acute invertebrate study based on OECD 202, US EPA OPPTS 850.1010 GLP	*Brachionus calyciflorus*	MITC, Purity: 98.9 %, Batch no.: A0337662	LC_{50} > 1.2 mg MITC/L (mean measured)	Acceptable Supportive study	24 h static 5 organisms/ replicate 6 replicates/ treatment	CA8.2.4.2/12 [redacted], 2014b
Acute invertebrate study based on OECD 225 and 202 GLP	*Lumbriculus variegatus*	MITC, Purity: ≥ 96.5 %, Batch no.: STBH5869	LC_{50} = 0.315 mg MITC/L (mean measured)	Acceptable Supportive study	96 h semi-static 5 organisms/ replicate 4 replicates/ treatment	CA8.2.4.2/13 [redacted]., 2019a
Acute invertebrate study based on OECD 242 and 202 GLP	*Potamopyrgus anitpodarum*	MITC, Purity: ≥ 96.5 %, Batch no.: STBH5869	LC_{50} = 0.319 mg MITC/L (mean measured)	Acceptable Supportive study	96 h semi-static 5 organisms/ replicate 4 replicates/ treatment	CA8.2.4.2/14 [redacted], 2019b
Acute invertebrate study based on OECD 202 GLP	*Dugesia tigrina*	MITC, Purity: ≥ 96.5 %, Batch no.:STBH5869	LC_{50} = 0.137 mg MITC/L (nominal)	Acceptable Supportive study	96 h semi-static 5 organisms/ replicate 4 replicates/ treatment	CA8.2.4.2/15 [redacted], 2019c
Acute invertebrate study based on OECD 202 and 235 GLP	*Crangonyx pseudogracilis*	MITC, Purity: 99.6 %, Batch no.:STBB1308V	LC_{50} = 0.312 mg MITC/L (mean measured)	Acceptable Supportive study	48 h semi-static 20 organisms/ replicate 1 replicate/ treatment	CA8.2.4.2/16 [redacted], 2014b
Acute invertebrate study based on OECD 202 and 235 GLP	*Lumbriculus variegatus*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	LC_{50} = 0.205 mg MITC/L (mean measured)	Acceptable Supportive study	48 h semi-static 5 organisms/ replicate 4 replicates/ treatment	CA8.2.4.2/17 [redacted], 2014c
Algae						
Algal growth inhibition study based on OECD 201 GLP	*Pseudokirchneriella subcapitata*	MITC, Purity: 99.0 %, Batch no.: 80525	E_bC_{50} = 0.281 mg MITC/L E_rC_{50} = 0.432 mg MITC/L (initial measured)	Acceptable Supportive study	72 h static Initial cell count: 1 x 10^4 cells/mL 5 replicates/ control 10 replicates/ solvent	CA8.2.6.1/05 [redacted] 1998 CA8.2.6.1/06 [redacted]

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
					control 5 replicates/ treatment	[redacted] 2019a
Algal growth inhibition study based on OECD 201, EU 92/69/EEC C Method C3, US EPA OPPTS 850.5400, ISO 14442 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_yC_{50} = 0.12 mg MITC/L E_yC_{10} = 0.08 mg MITC/L E_rC_{50} = 0.21 mg MITC/L E_rC_{10} = 0.09 mg MITC/L $NOAEC_y$ = 0.044 mg MITC/L $NOAEC_r$ = 0.11 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/07 [redacted], 2012d
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} = 0.189 mg MITC/L E_rC_{10} = 0.076 mg MITC/L E_yC_{50} = 0.091 mg MITC/L E_yC_{10} = 0.051 mg MITC/L E_bC_{50} = 0.0933 mg MITC/L E_bC_{10} = 0.0456 mg MITC/L (mean measured)	Acceptable Key study	72 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/09 [redacted], 2018b CA8.2.6.1/10 [redacted] 2020d
Algal growth inhibition study based on ASTM E 1218-90,	*Anabaena flos-aquae*	MITC, Purity: 99.6 %, Batch no.: 408208/1	E_rC_{50} = 3.607 mg MITC/L E_rC_{10} = 1.143 mg MITC/L	Acceptable Supportive study	72 h static Initial cell count: 3 x 10^4 cells/mL 10 replicates/	CA8.2.6.2/01 [redacted]., 2002 CA8.2.6.2/02 [redacted].

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
OECD 201, OPPTS 850.1000 GLP			E_yC_{50} = 1.886 mg MITC/L E_yC_{10} = 0.793 mg MITC/L (initial measured)		control 5 replicates/ treatment	[redacted], 2019b
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPPTS 850.5400, ISO 14442 GLP	*Anabaena flos-aquae*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_rC_{50} (72 h) = 0.433 mg MITC/L E_yC_{50} (72 h) = 0.431 mg MITC/L E_bC_{50} (96 h) = 0.1792 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/03 [redacted], 2012a CA8.2.6.2/04 [redacted], 2020
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPTS 850.5400, ISO 14442 GLP	*Navicula pelliculosa*	MITC, Purity: 99.7 %, Batch no.:56198PJV	E_rC_{50} (72 h) = 0.349 mg MITC/L E_rC_{10} (72 h) = 0.144 mg MITC/L E_yC_{50} (72 h) = 0.181 mg MITC/L E_bC_{50} (72 h) = 0.1804 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 8 replicates/ control 4 replicates/ treatment	CA8.2.6.2/05 [redacted] 2012b CA8.2.6.2/06 [redacted], 2020a
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPTS	*Skeletonema costatum*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_rC_{50} (72 h) > 0.430 mg MITC/L E_rC_{10} (72 h) = 0.0351 mg MITC/L E_yC_{50} (72	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/07 [redacted], 2012c CA8.2.6.2/08 [redacted], 2020b

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
850.5400, ISO 14442 GLP			h) = 0.081 mg MITC/L E_yC_{10} (72 h) = 0.0246 mg MITC/L E_bC_{50} (72 h) = 0.1391 mg MITC/L E_bC_{10} (72 h) = 0.021 mg MITC/L (initial measured)			
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3	*Anabaena flos-aquae*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (72 h) = 0.375 mg MITC/L E_rC_{10} (72 h) = 0.173 mg MITC/L E_yC_{50} (72 h) = 0.181 mg MITC/L E_yC_{10} (72 h) = 0.051 mg MITC/L E_bC_{50} (72 h) = 0.208 mg MITC/L E_bC_{10} (72 h) = 0.067 mg MITC/L (nominal)	Acceptable Supportive study	72 h static Initial cell count: 10000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/09 [redacted], 2019 CA8.2.6.2/10 [redacted]., 2020e
Lemna growth inhibition study based on OECD 221, OPPTS 850.5400, ASTM E1415-91,	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: 408208/1	E_rC_{50} = 1.133 mg MITC/L E_rC_{10} = 0.411 mg MITC/L E_bC_{50} = 0.556 mg MITC/L	Endpoint is uncertain Not acceptable for classification	7 d semi-static Inoculation with one plant with 4 fronds and two plants with 3 fronds, 6 replicates/	CA8.2.7/01 [redacted], 2002 CA8.2.7/02 [redacted]., 2019d

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
EPA J, 132-2			E_bC_{10} = 0.160 mg MITC/L (mean measured)		control 3 replicates/ treatment	
Lemna growth inhibition study based on OECD 221, EC 2016/266 Method C26	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (frond numbers) = 0.43 mg MITC/L E_rC_{10} (frond numbers) = 0.18 mg MITC/L E_rC_{50} (dry weight) = 0.29 mg MITC/L E_rC_{10} (dry weight) = 0.13 mg MITC/L (mean measured)	Acceptable Key study	7 d flow-through Inoculation with 3 randomly selected colonies per vessel (12 fronds/ 3 colonies) 3 replicates/ control 3 replicates/ solvent control 3 replicates/ treatment	CA8.2.7/03 [redacted], 2019c

2.9.2.2.1 Acute (short-term) toxicity to fish

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. 8 acute (short-term) toxicity studies with fish are available for metam (performed with either metam-sodium or metam-potassium), and 5 such studies are available for MITC.

Studies with metam

Data point:	KCA 8.2.1/01
Report author:	[redacted]
Report year:	1992
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-1
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Water temperature: 11.6 °C – 15.4 °C (recommendation: 10 °C – 14 °C for *Oncorhynchus mykiss*) Water temperature changes per day: 2.9 °C (0 h – 24 h), 3.7 °C (72 h – 96 h) (recommendation: 2 °C) Feeding withdrawal before exposure: 72 hours (recommendation: 24 – 4 hours)

	Deviations are not considered significant and did not affect the results of the test.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Buckman Laboratories International)

Study Summary:

In a flow-through acute toxicity test, Rainbow trout (*Oncorhynchus mykiss*) were exposed for 96 hours to 5 nominal concentrations ranging between 13.5 and 104 mg Potassium N-methyldithiocarbamate (metam-potassium)/L water and a water control. Actual measured concentrations of metam-potassium were between 13.3 and 106 mg metam-potassium/L (96 – 102 % recovery) and therefore in good agreement with the nominal concentrations.
Mortality of Rainbow trout exposed for 96 hours to metam-potassium ranged from 0 % at mean measured concentrations of ≤ 21.7 mg metam-potassium/L to 100 % at 106 mg metam-potassium/L. Mortality in the dilution water control was 0 %. Sublethal effects were observed at all test concentrations. Fish exposed to different concentrations of test item exhibited clinical symptoms such as reduced activity, orientation to bottom or surface of the test vessels and dark pigmentation.
The 96-hour LC_{50} based on measured concentrations was 62.4 mg metam-potassium/L with 95 % confidence limits of 54.4 to 71.3 mg metam-potassium/L. Although no mortality occurred at 13.3 or 21.7 mg metam-potassium/L, a no-observed-effect concentration (NOEC) could not be determined because of sublethal effects observed at these test concentrations.

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	juveniles, mean weight at start: 0.36 ± 0.12 g, mean length at start: 37 ± 4.0 mm, fish loading: 0.06 g fish/L
Acclimatisation of the fish:	3 weeks acclimatisation, no feeding during the test
Type of test:	Flow-through toxicity test (15 L of test solution with 7.6 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 13.5, 22.5, 37.4, 62.4 and 104 mg a.s./L
Number of animals per group:	20 fish for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 11.6 – 15.4 °C dissolved oxygen: 8.1 – 10.2 mg/L O_2, 75 – 94 % O_2 saturation pH: 8.2 – 8.5 hardness: 168 mg/L $CaCO_3$ (start), 152 mg/L $CaCO_3$ (end) alkalinity: 101 mg/L $CaCO_3$ (start), 98 mg/L $CaCO_3$ (end) photoperiod: 16 hours light and 8 hours dark light intensity: 298 – 383 lux

Test procedure:	In a flow-through freshwater toxicity test, juvenile Rainbow trout were exposed by groups of 20 animals per treatment for 96 hours to the following nominal concentrations: 0 (negative control), 13.5, 22.5, 37.4, 62.4 and 104 mg metam-potassium/L. Concentrations for the main test were defined in a preliminary range-finder test. Fish loading was 0.06 grams of tissue per liter of test solution.
Test item analysis:	Pre-test samples were collected from the low, middle, and high test concentrations to verify expected nominal concentrations and proper diluter function. Water samples were collected from the control and each metam-potassium test solution on test days 0, 2 and 4 to monitor actual exposure concentrations. Quantitation of metam-potassium was performed by high performance liquid chromatography (HPLC) using an UV-VIS detector and the external standard technique.
Observations:	Survival of fish was monitored daily and any dead removed when observed. Any abnormalities in the behaviour or physical appearance of the fish were also noted. Test water quality was monitored daily.
Statistical evaluation:	Based on results of the test, the 24-, 48-, 72- and 96-hour LC_{50} values and their 95 % confidence limits were calculated. The LC_{50} values were estimated by a computer program using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for LC_{50} values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0-% and 100-% mortality and the number of concentrations in which mortalities between 0 and 100 % occurred.

Findings:

Analytical results:	Test concentrations were 93 to 97 % of nominal at test initiation and remained stable throughout the test. Actual measured concentrations of metam-potassium were between 13.3 and 106 mg a.s./L (96 – 102 % recovery). Test solutions remained clear throughout the test and no precipitation of test substance was observed. The measured concentrations in aged samples (and *a fortiori* in fresh samples) are between 80 and 120 % of nominal, however, the following results for mortality are presented for measured concentrations of metam-potassium.
Mortality:	Mortality of Rainbow trout exposed for 96 hours to metam-potassium ranged from 0 % at mean measured concentrations of ≤ 21.7 mg a.s./L to 100 % at 106 mg a.s./L. Mortality in the dilution water control was 0 %. The 96-hour LC_{50} based on measured metam-potassium concentrations was 62.4 mg a.s./L with 95 % confidence limits of 54.4 to 71.3 mg a.s./L.
Behaviour and clinical signs:	Although no mortality occurred at 13.3 or 21.7 mg a.s./L, a no-observed-effect concentration (NOEC) could not be determined because of sublethal effects observed at these test concentrations. Details are presented in the table below.

Table B.2.9.2-1: Mortality of Rainbow trout (*Oncorhynchus mykiss*), exposed to metam-potassium for 96 hours under flow-through test conditions

Nominal concentration (mg metam-potassium/L)	Mean measured concentration (mg metam-potassium/L)	Mortality (% mortality)			
		24 hours	48 hours	72 hours	96 hours
control	-	0 (0)	0 (0)	0 (0)	0 (0)
13.5	13.3	0 (0)[a]	0 (0)[f]	0 (0)	0 (0)
22.5	21.7	0 (0)[b]	0 (0)[g]	0 (0)	0 (0)[g]
37.4	37.0	0 (0)[c]	0 (0)[h]	1 (5)[k]	1 (5)[m]
62.4	63.0	0 (0)[d]	0 (0)[i]	5 (25)[l]	9 (45)[n]
104.0	106.0	2 (10)[e]	15 (75)[j]	20 (100)	20 (100)

[a] Four fish on bottom, one dark. [b] Two fish on bottom, all fish darker than control fish. [c] Six fish on bottom, all dark. [d] Twelve fish on bottom, all dark. [e] Two with complete loss of equilibrium, all dark.[f] Two fish dark, five resting on bottom. [g] Three fish dark. [h] One fish lying on the bottom, all fish dark. [i] Two fish lying on the bottom, all other fish resting upright on the bottom. All fish dark, three with patches of lighter pigment. [j] All fish lying on the bottom. All fish dark with large patches of lighter or no pigment areas. [k] Ten fish dark. [l] Seven fish with patches. All fish dark. [m] Three fish immobile, sixteen fish dark and on bottom. [n] Ten fish immobile with loss of pigment, one fish dark.

Assessment and conclusions:

The 96 hours LC_{50} of metam-potassium to the Rainbow trout (*Oncorhynchus mykiss*) was 62.4 mg a.s./L with 95 % confidence limits of 54.4 to 71.3 mg a.s./L (based on mean measured concentrations). Although no mortality occurred at 13.3 and 21.7 mg a.s./L, a NOEC could not be determined due to observed sublethal effects.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoint:

LC_{50} (*Oncorhynchus mykiss*, 96 h) = 62.4 mg metam-potassium/L

Analytical method:

Despite some minor deviations from the guidance SANCO/3029/99 rev. 4 on analytical validation, the method is assessed to be fit for purpose, and therefore considered acceptable as supplementary data.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to fish in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The validity criteria of OECD Guideline 203 were met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: 75 – 78 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.

The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered “fit for purpose” (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/42, for further details).

LC_{50} (*Oncorhynchus mykiss*, 96 h, flow-through) = 62.4 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Note of RMS: In the summary dossier of the applicant Eastman, the endpoint LC_{50} was mentioned to be based on nominal concentrations. However, RMS assessment of the original study report confirms the endpoint LC_{50} to be based on mean measured concentrations.

Data point:	KCA 8.2.1/02
Report author:	[redacted]
Report year:	1971
Report title:	Vapam: Safety Evaluation on Rainbow Trout.
Report No.:	[redacted]
Document No.:	[redacted]
Guidelines followed in study:	None
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Analytical determinations and measurements: Not performed Last feeding: 72 hours before test start (recommendation: 24 – 48 hours before last feeding) Reporting: No detailed information on test material, environmental conditions like pH values or dissolved oxygen concentrations No test item analysis was performed (minimum requirement: analysis of the highest and lowest test concentration and a concentration around the expected LC_{50}) LC_{50} values were determined after 48 and 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours)
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Stauffer Chemical Company)

Study Summary:

In a static acute toxicity test Rainbow trout (*Oncorhynchus mykiss*) were exposed to a dilution water control and Vapam nominal concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L (corresponding to 0.0327, 0.0589, 0.105, 0.183 and 0.327 mg sodium methyl dithiocarbamate (metam-sodium)/L). Each treatment group consisted of 1 or 2 replicates, each containing 10 fish. No mortality or signs of intoxication were observed in the control group. The nominal based LC_{50} value for Vapam after 48 hours was 0.26 mg/L (95% confidence limits: 0.22 – 0.31 mg/L), corresponding to 0.085 mg metam-sodium/L (95% confidence limits: 0.072 – 0.101 mg metam-sodium/L). After 96 hours LC_{50} value was 0.24 mg/L

(95% confidence limits: 0.21 – 0.27 mg/L), corresponding to 0.0785 mg metam-sodium/L (95% confidence limits: 0.069 – 0.088 mg metam-sodium/L).

Materials and methods:

Test substance:	Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch no: not reported
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 0.73 g, mean length at start: 4.3 cm, fish loading: 0.24 g fish/L
Acclimatisation of the fish:	minimum 10 days acclimatisation, no feeding during the test
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.10, 0.18, 0.32, 0.56 and 1.0 mg Vapam/L corresponding to 0, 0.0327, 0.0589, 0.105, 0.183 and 0.327 mg a.s./L
Number of animals per group:	10 fish per replicate, 2 replicates for the control and per treatment group (1 replicate for treatment level 0.10 mg Vapam/L)
Time of exposure:	96 hours
Test conditions:	temperature: 11 – 12 °C dissolved oxygen: not reported pH: not reported hardness: not reported alkalinity: not reported photoperiod: not reported light intensity: not reported
Test procedure:	In a static acute toxicity test Rainbow trout (*Oncorhynchus mykiss*) were exposed to a dilution water control and Vapam nominal concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L (corresponding to 0.0327, 0.0589, 0.105, 0.183 and 0.327 mg sodium methyl dithiocarbamate (metam-sodium)/L). Therefore, the test material was dissolved in distilled water and aliquots required to attain the desired concentrations were pipetted to jars containing 15 liters. Each treatment group consisted of 2 replicates, each containing 10 fish.
Test item analysis:	No test item analysis was performed.
Observations:	Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. LC_{50} was determined after 48 and 96 hours and refers to nominal concentrations.
Statistical evaluation:	Data were examined by probit analysis or the simplified method of Litchfield and Wilcoxon.

Findings:

Analytical results:	No test item analysis was performed.
Mortality:	No mortalities occurred in the control group or at the treatment level of 0.10 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 0.18, 0.32, 0.56 and 1.0 mg Vapam/L after 96 hours of exposure was 5 %, 95 %, 100 % and 100 %, respectively. The nominal based LC_{50} value for Vapam after 48 hours was 0.26 mg/L (95 % confidence limits: 0.22 – 0.31

mg/L), corresponding to 0.085 mg metam-sodium/L (95 % confidence limits: 0.072 – 0.101 mg metam-sodium/L). The nominal based LC_{50} value for Vapam after 96 hours was 0.24 mg/L (95 % confidence limits: 0.21 – 0.27 mg/L), corresponding to 0.078 mg metam-sodium/L (95 % confidence limits: 0.069 – 0.088 mg metam-sodium/L).

Behaviour and clinical signs: No signs of intoxication were observed in the control group. Signs of intoxication were seen after 24 hours at Vapam nominal concentrations of 1.0, 0.56 and 0.32 mg/L (corresponding to 0.105, 0.183 and 0.327 mg metam-sodium/L) and consisted of fish swimming near the surface accompanied by a slight loss of equilibrium and side-swimming. These signs appeared within two hours after Vapam exposure at 1.0 mg/L (0.327 mg metam-sodium/L) and from 24 to 48 hours at 0.56 and 0.32 mg/L (0.105 and 0.183 mg metam-sodium/L, respectively).

Table B.2.9.2-2: Mortality of Rainbow trout (*Oncorhynchus mykiss*), exposed to Vapam (metam-sodium) for 96 hours under static test conditions

Nominal concentration of test item (mg Vapam/L)	Number of fish	Mortality at				Cumulative mortality [%]
		24 hours	48 hours	72 hours	96 hours	
control	20	0/20	0/20	0/20	0/20	0
0.10	10	0/10	0/10	0/10	0/10	0
0.18	20	0/20	0/20	1/20	1/20	5
0.32	20	2/20	16/20	19/20	19/20	95
0.56	20	11/20	19/20	20/20	20/20	100
1.0	20	20/20	20/20	20/20	20/20	100

Assessment and conclusions:

Results of this investigation indicate that Vapam has a nominal 96 hour LC_{50} of 0.24 mg/L (95 % confidence limits: 0.21 – 0.27 mg/L) in Rainbow trout (*Oncorhynchus mykiss*). This LC_{50} corresponds with to 0.0785 mg metam-sodium/L (95 % confidence limits: 0.069 – 0.088 mg metam-sodium/L).

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is submitted for information.

The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoint:

LC_{50} (*Oncorhynchus mykiss*, 96 h) = 0.24 mg Vapam/L = 0.0785 mg metam-sodium/L (nominal)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is mentioned in the dossier of the applicant Lainco.

EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :

LC_{50} (*Oncorhynchus mykiss*, 96 h, static) = 0.24 mg Vapam/L = 0.078 mg metam-sodium/L (nominal)

Lainco S.A. notes that no analytical verification of test concentrations was included and hence the study is reported here for supporting information only, on request from the RMS during the completeness check.

Assessment and conclusion by the RMS:
The study is compared with the current guidance.
The validity criteria of OECD Guideline 203 were not all met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (not reported)
- analytical measurement of test concentrations is compulsory (not conducted)

The study shows a lot of shortcomings in comparison to current guidance, however it gives a good indication of the toxicity level of metam-sodium.
LC_{50} (*Oncorhynchus mykiss*, 96 h, static) = 0.24 mg Vapam/L = 0.078 mg metam-sodium/L (based on nominal concentrations)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/03
Report author:	[redacted]
Report year:	1986
Report title:	Report on the Study of Acute Toxicity of METAM-Sodium in the Bluegill (*Lepomis macrochirus Raf.*)
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-1, 1982
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Reporting: No detailed information on test chemical and test item analysis. No information on preparation of stock solutions. Acclimation period: 5 days (recommendation: 48 hours settling-in + 7 days acclimatisation = 9 days) Temperature was not measured continuously in one tank as recommended.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF Corporation Chemicals Division)

Study Summary:

Based on the results of a range finding test Bluegill sunfish (*Lepomis macrochirus*) was exposed to metam-sodium nominal concentrations of 0.1, 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg/L in a static acute toxicity test for 96 hours according to U.S. EPA Guideline OPPS 72-1. 10 animals per concentration were tested with a loading rate of 0.04 g/L. Mortality of Bluegill sunfish exposed for 96 hours to metam-sodium ranged from 0 % at nominal concentrations of 0.1 mg metam-sodium/L to 100 %

at 0.464 mg metam-sodium/L and higher concentrations. The derived nominal based 96 hour LC_{50} value was > 0.464 mg/L (5 % significance level) and < 1.000 mg/L (1 % significance level).

Materials and methods:

Test substance:	Metam-sodium, batch no: ZH 130 585, chemical purity: 42.2 %
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 0.4 g, mean length at start: 3.3 cm, fish loading: 0.04 g fish/L
Acclimatisation of the fish:	3 – 5 days acclimatisation, no feeding during the test
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.100, 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg a.s./L
Number of animals per group:	10 fish for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 22 – 23 °C dissolved oxygen: 6.0 – 8.8 mg/L O_2, 69 – 101 % O_2 saturation pH: 7.5 – 8.1 hardness: 2.5 mmol/L alkalinity: not reported photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	Based on the results of a range finding test (LC_{50} of 3 mg/L) Bluegill sunfish (*Lepomis machrochirus*) were exposed for 96 hours to nominal concentrations of 0.1, 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg/L. The test item was added as an aqueous solution to the test water. Subsequently the fish were placed into the aquaria. 10 animals per concentration were tested with a loading rate of 0.04 g/L.
Test item analysis:	Metam-sodium concentrations were determined by HPLC at test initiation in all treatment groups and in the 0.100, 0.215, 0.464 and 1.00 mg/L treatment groups at test termination.
Observations:	Mortality and other symptoms were determined after 1, 4, 24, 48, 72 and 96 hours. Temperature, dissolved oxygen concentration and pH were measured at the beginning of the test and after 24, 48, 72 and 96 hours.
Statistical evaluation:	Median lethal concentration (LC_{50}) and, if possible, the LC_5 and the LC_{95} were calculated using Probit analysis.

Findings:

Analytical results:	Test concentrations were 52 to 98 % of nominal at test initiation. After 96 hours measured metam-sodium concentrations of the 0.100, 0.215, 0.464 and 1.00 mg/L treatment groups were 70 %, 81 %, 20 % and 82 % of nominal concentrations and between 23.8 % and 135 % of initially measured concentrations.
Mortality:	Mortality of Bluegill sunfish exposed for 96 hours to metam-sodium ranged from 0 % at nominal concentrations of 0.1 mg a.s./L to 100 % at ≥ 0.464 mg a.s./L. Detailed results of mortality and measured concentrations are presented in the table below.

Behaviour and clinical signs: No clinical signs of toxicity were observed at the treatment levels of 0.100 and 0.215 mg a.s./L. At the treatment level of 21.5 mg a.s./L, clinical signs of toxicity observed after 48 hours of exposure were apathy and abnormal swimming behaviour.

Table B.2.9.2-3: Mortality of Bluegill sunfish (*Lepomis macrochirus*), exposed to metam-sodium for 96 hours under static test conditions

Nominal concentration of test item (mg a.s./L)	Initial measured concentration (mg a.s./L)	% of nominal	Measured concentration 96 hours (mg a.s./L)	% of initial measured	Number of fish	Number of dead	
						48 hours	96 hours
0.000	-	-	-	-	10	0	0
0.100	0.052	52	0.07	135	10	0	0
0.215	0.194	90	0.175	90.2	10	0	0
0.464	0.40	86	0.095	23.8	10	0	1
1.00	0.75	75	0.82	109	10	6	10
2.15	1.76	82	n.m.	-	10	10	10
4.64	4.36	94	n.m.	-	10	9	10
10.0	9.76	98	n.m.	-	10	10	10
21.5	18.95	90	n.m.	-	10	1	10

n. m. = not measured

Calculated effect concentrations based on nominal concentrations were as follows:

Nominal concentrations LC_{50} > 0.464 mg a.s./L (5% significance level)
LC_{50} < 1.000 mg a.s./L (1% significance level)

Calculated effect concentrations based on concentrations measured at test termination were as follows:

Concentrations measured at 96 h: LC_{50} > 0.175 mg a.s./L (1% significance level)
LC_{50} < 0.820 mg a.s./L (1% significance level)

Assessment and conclusions:

Bluegill sunfish (*Lepomis macrochirus*) were exposed for 96 hours to nominal concentrations of 0.1, 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg metam-sodium/L. The 96 hour LC_{50} value was > 0.464 mg a.s./L and < 1.000 mg a.s./L (nominal).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Endpoints:
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.464 mg metam-sodium/L (nominal)
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.175 mg metam-sodium/L (measured at 96 hours)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.175 mg metam-sodium/L (measured at 96 hours)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: 69 - 101 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.
No further information on the analytical method used.
LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.464 mg metam-sodium/L (based on nominal concentrations)
LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.175 mg metam-sodium/L (based on measured concentrations at 96 hours)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.1/04.

Data point:	KCA 8.2.1/04
Report author:	[redacted]
Report year:	2019a
Report title:	Report on the Study of Acute Toxicity of METAM-Sodium in the Bluegill (*Lepomis macrochirus Raf.*) – Statistical Re-analysis.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 210: "Fish Early Life Stage Toxicity Test", adopted July 26 (Annex 6), 2013
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Due to some limitations in the main study (KCA 8.2.1/03, [redacted], 1986) compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report by [redacted], project number 86/0512 ([redacted], 1986; KCA 8.2.1/03); for the toxicity of METAM-Sodium to Bluegill sunfish (*Lepomis Macrochirus Raf.*), did not provide estimates of the LC_{10}, LC_{20} or LC_{50} for the response variable (mortality) evaluated as part of the original

study. Consequently the data generated in this study were intended to be re-analysed in an attempt to provide these values.
The test design consisted of seven concentrations of the test substance (nominally 0.1, 0.215, 0.464, 1.0, 2.15, 4.64, 10 and 21.5 mg test item/L and a control). The mean measured concentrations after 1 hour were within ± 20 % of the nominal values with the exception of the 0.1 and 1 mg/L treatment groups where 52 and 75 % of the nominal values were observed, respectively. After 96 hours, chemical analysis was only reported for the nominal 0.1, 0.215, 0.464 and 1 mg/L treatment groups. The mean measured values after 96 hours ranged from 70 – 82 % with the exception of the nominal 0.464 mg/L treatment group where only 20 % recovery was observed. Mean measured concentrations in the original report were determined as 0.052, 0.194, 0.4, 0.75, 1.76, 4.36, 9.76 and 18.95 mg/L. In line with the original report, these analyses are conducted using both the nominal and mean measured concentrations.
Statistical analysis of the available data for **survival** revealed that the following LC_{10}, LC_{20} and LC_{50} values were reliably calculated using the mean measured values:

Table B.2.9.2-4: Mortality of Bluegill Sunfish (*Lepomis macrochirus*), exposed to metam-sodium for 96 hours under static test conditions, statistical re-analysis based on mean measured values

Parameter	Survival using mean measured values		
	LC_{10}	LC_{20}	LC_{50}
Value [mg/L]	0.296	0.359	0.522
lower 95 %-cl	0.201	0.262	0.414
upper 95 %-cl	0.377	0.449	0.654

No reliable LC_x values could be determined using the nominal concentrations.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Statistical recalculation of endpoints for the study KCA 8.2.1/03 ([redacted]., 1986)
Original Endpoints:
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.464 mg metam-sodium/L (nominal)
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.175 mg metam-sodium/L (measured at 96 hours)
Endpoints from re-analysis:
No reliable LC_x values could be determined using the nominal concentrations.
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.522 mg metam-sodium/L (mean measured)
Assessment and conclusion by Lainco:
The statistical re-analysis is not mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Lepomis macrochirus*, 96 h) > 0.175 mg metam-sodium/L (measured at 96 hours)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: 69 - 101 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table under KCA 8.2.1/03)

Therefore, this study is still considered acceptable.
No further information on the analytical method used.
LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.464 mg metam-sodium/L (based on nominal concentrations)

LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.175 mg metam-sodium/L (based on measured concentrations at 96 hours) A statistical re-analysis of the original endpoints was conducted: No reliable LC_x values could be determined using the nominal concentrations. LC_{50} (*Lepomis macrochirus*, 96 h, static) > 0.522 mg metam-sodium/L (mean measured) Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/05
Report author:	[redacted]
Report year:	1992
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute Toxicity To Bluegill, *Lepomis macrochirus*, Under Flow-Through Test Conditions.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-1
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Test temperature: 20.4 – 21.7 °C (recommendation: 21 – 25 °C) Temperature variation in a 24 h period (1.1 °C) > 1.0 °C Length of fish: 30 ± 4 mm (recommendation: 10 – 30 mm) No signs of stress were observed with control fish. The protocol deviation is not considered significant, and did not affect the test results.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Buckman Laboratories International)

Study Summary:

In a flow-through freshwater toxicity test, juvenile Bluegill sunfish (*Lepomis macrochirus*) were exposed in two replicates of 10 animals per treatment for 96 hours to the following nominal concentrations: 0 (negative control), 15.6, 26.0, 43.2, 72.0 and 120 mg Potassium N-methyldithiocarbamate (metam-potassium)/L. Actual mean measured test concentrations were 98 to 103 % of nominal at test initiation and remained stable throughout the test. Mean measured concentrations of metam-potassium ranged from 16.7 mg a.s./L to 125 mg a.s./L and from 103 to 107 % of nominal. Test solutions remained clear throughout the test and no precipitation of test substance was observed. The 96 hour LC_{50} based on measured metam-potassium concentrations was 108 mg a.s./L with 95 % confidence limits of 95.3 to 127 mg a.s./L. Although no mortality occurred at test concentrations 16.7, 26.7 and 45.9 mg a.s./L, a no-observed-effect concentration NOEC could not be determined because of sublethal effects observed at those test concentrations.

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Age, weight, length, loading:	juveniles, mean weight at start: 0.77 ± 0.26 g, mean length at start: 30 ± 4 mm, fish loading: 0.14 g fish/L
Acclimatisation of the fish:	Acclimatisation period: not reported, no feeding during the test
Type of test:	Flow-through toxicity test (15 L of test solution with 7.4 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 15.6, 26.0, 43.2, 72.0 and 120 mg a.s./L
Number of animals per group:	10 fish per replicate, 2 replicates for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 20.4 – 21.7 °C dissolved oxygen: 7.2 – 8.4 mg/L O_2 (start), 80 – 93 % O_2 saturation pH: 8.1 – 8.4 hardness: 124 mg/L $CaCO_3$ (start), 96 mg/L $CaCO_3$ (end) alkalinity: 94 mg/L $CaCO_3$ (start), 84 mg/L $CaCO_3$ (end) photoperiod: 16 hours light and 8 hours dark light intensity: 318 – 511 lux
Test procedure:	In a flow-through freshwater toxicity test, juvenile Bluegill sunfish were exposed in two replicates of 10 animals per treatment for 96 hours to the following nominal concentrations: 0 (negative control), 15.6, 26.0, 43.2, 72.0 and 120 mg metam-potassium/L. Concentrations for the main test were defined in a preliminary range-finder test. Fish loading was 0.14 grams of tissue per liter of test solution.
Test item analysis:	Pre-test samples were collected from the low, middle, and high test concentrations to verify expected nominal concentrations and proper diluter function. Water samples were collected from the control and each metam-potassium test solution on test days 0, 2 and 4 to monitor actual exposure concentrations. Water samples (approximately 10 mL in volume) were pipetted from midway in the water column. Quantitation of metam-potassium was performed by high performance liquid chromatography (HPLC) using an UV-VIS detector and the external standard technique.
Observations:	Survival of fish was monitored daily and any dead fish were removed when observed. Any abnormalities in the behavior or physical appearance of the fish were also noted. Test water quality was monitored daily.
Statistical evaluation:	The LC_{50} values were estimated by a computer program using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for LC_{50} values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0 % and 100 % mortality and the number of concentrations in which mortalities between 0 and 100 % occurred.

Findings:

Analytical results:	Test concentrations were 98 to 103 % of nominal at test initiation and remained stable throughout the test. Mean measured concentrations of metam-potassium ranged from 16.7 mg a.s./L to 125 mg a.s./L and from 103 to 107 % of nominal. Test solutions remained clear throughout the test and no precipitation of test substance was observed.
Mortality:	Mortality of Bluegill sunfish exposed for 96 hours to metam-potassium ranged from 0 % at mean measured concentrations of ≤ 45.9 mg a.s./L to 70 % at 125 mg a.s./L. Mortality in the dilution water control was 0 %. Detailed results of cumulative mortality are presented in the table below.
Behaviour and clinical signs:	Although no mortality occurred at 16.7, 26.7 and 45.9 mg a.s./L, a no-observed-effect concentration (NOEC) could not be determined because of sublethal effects observed at these test concentrations. Clinical signs of toxicity observed were dark pigmentation in the head region and on the entire fish. Detailed results of clinical signs are presented in the table below.

Table B.2.9.2-5: Mortality and clinical signs of Bluegill sunfish (*Lepomis macrochirus*), exposed to metam-potassium for 96 hours under flow-through test conditions

Nominal concentration of test item (mg a.s./L)	Mean measured concentration of test item (mg a.s./L)	Number of fish	Cumulative number of dead (% mortality)			
			24 hours	48 hours	72 hours	96 hours
Control	-	20	0 (0)	0 (0)	0 (0)	0 (0)
15.6	16.7	20	0 (0)	0 (0)	0[a] (0)	0 (0)
26.0	26.7	20	0 (0)	0 (0)	0[a] (0)	0 (0)
43.2	45.9	20	0 (0)	0 (0)	0[a] (0)	0 (0)
72.0	76.5	20	1 (5)	1 (5)	2[b] (10)	2[b] (10)
120	125	20	2 (10)	3 (15)	8[b] (40)	14[b] (70)

[a] dark pigmentation in head region; [b] dark pigmentation on entire fish

Assessment and conclusions:

The 96 hour LC_{50} for Bluegill sunfish (*Lepomis macrochirus*) based on measured metam-potassium concentrations was 108 mg a.s./L with 95 % confidence limits of 95.3 to 127 mg a.s./L. Although no mortality occurred at test concentrations 16.7, 26.7 and 45.9 mg a.s./L, a no-observed-effect concentration NOEC could not be determined because of sublethal effects observed at those test concentrations.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable as supplementary data.

Endpoint:

LC_{50} (*Lepomis macrochirus*, 96 h) = 108 mg metam-potassium/L (mean measured)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to fish in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: 80 – 93 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/43, for further details).
LC_{50} (*Lepomis macrochirus*, 96 h, flow-through) = 108 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/06
Report author:	[redacted].
Report year:	1970a
Report title:	Vapam: Safety Evaluation on Bluegill Sunfish.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	None
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Analytical determinations and measurements: Not performed Last feeding: 72 hours before test start (recommendation: 24 – 48 hours before last feeding) Reporting: No detailed information on test material, environmental conditions like pH values or dissolved oxygen concentrations No test item analysis was performed (minimum requirement: analysis of the highest and lowest test concentration and a concentration around the expected LC_{50}) LC_{50} values were determined after 48 and 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours) Temperature not in recommended range (21 – 25 °C), actually 16.7 – 17.8 °C
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)

Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Stauffer Chemical Company)

Study Summary:

In a static acute toxicity test Bluegill sunfish (*Lepomis machrochirus*) were exposed to a dilution water control and Vapam nominal concentrations of 0.56, 1.0, 1.4, 1.8, 3.2 and 10.0 mg/L (corresponding to 0.183, 0.327, 0.458, 0.589, 1.05 and 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L). Each treatment group consisted of 1 or 2 replicates, each containing 10 fish. No mortality or signs of intoxication were observed in the control group. The nominal based LC_{50} value for Vapam after 48 hours was 1.73 mg/L (95 % confidence limits: 1.55 – 2.03 mg/L), corresponding to 0.566 mg metam-sodium/L (95 % confidence limits: 0.507 – 0.664 mg metam-sodium/L). After 96 hours LC_{50} value was 1.19 mg/L (95 % confidence limits: 1.08 – 1.30 mg/L), corresponding to 0.389 mg metam-sodium/L (95 % confidence limits: 0.353 – 0.425 mg metam-sodium/L).

Materials and methods:

Test substance:	Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch no: not reported
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 2.52 g, mean length at start: 5.1 cm, fish loading: 0.84 g fish/L
Acclimatisation of the fish:	minimum 10 days acclimatisation, no feeding during the test
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.56, 1.0, 1.4, 1.8, 3.2 and 10.0 mg Vapam/L corresponding to 0, 0.18, 0.33, 0.46, 0.59, 1.05 and 3.27 mg a.s./L
Number of animals per group:	5 fish per replicate, 4 replicates for the control and for the treatment groups of 1.0, 1.4 and 1.8 mg Vapam/L, 2 replicates for the treatment groups of 0.56, 3.2 and 10.0 mg Vapam/L
Time of exposure:	96 hours
Test conditions:	temperature: 16.7 – 17.8 °C dissolved oxygen: not reported pH: not reported hardness: not reported alkalinity: not reported photoperiod: not reported light intensity: not reported
Test procedure:	In a static acute toxicity test Bluegill sunfish (*Lepomis machrochirus*) were exposed to a dilution water control and Vapam nominal concentrations of 0.56, 1.0, 1.4, 1.8, 3.2 and 10.0 mg/L (corresponding to 0.183, 0.327, 0.458, 0.589, 1.05 and 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L). Therefore, the test material was dissolved in distilled water and aliquots required to attain the desired concentrations were pipetted to jars containing

	15 liters. Each treatment group consisted of 1 or 2 replicates, each containing 10 fish.
Test item analysis:	No test item analysis was performed.
Observations:	Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. LC_{50} was determined after 48 and 96 hours and refers to nominal concentrations.
Statistical evaluation:	Data were examined by probit analysis.

Findings:

Analytical results:	No test item analysis was performed.
Mortality:	No mortalities occurred in the control group or at the treatment level of 0.56 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 1.0, 1.4, 1.8, 3.2 and 10.0 mg Vapam/L after 96 hours of exposure was 20 %, 75 %, 100 %, 100 % and 100 %, respectively. The nominal based LC_{50} value for Vapam after 48 hours was 1.73 mg/L (95 % confidence limits: 1.55 – 2.03 mg/L), corresponding to 0.566 mg metam-sodium/L (95 % confidence limits: 0.507 – 0.664 mg metam-sodium/L). The nominal based LC_{50} value for Vapam after 96 hours was 1.19 mg/L (95 % confidence limits: 1.08 – 1.30 mg/L), corresponding to 0.389 mg metam-sodium/L (95 % confidence limits: 0.353 – 0.425 mg metam-sodium/L).
Behaviour and clinical signs:	Signs of intoxication were seen at levels of 1.0, 1.4, 1.8, 3.2 and 10.0 mg/L (corresponding to 0.327, 0.458, 0.589, 1.05 and 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L). Those signs consisted of fish swimming near the surface accompanied by a slight loss of equilibrium and side swimming. These signs appeared within four hours after exposure at 3.2 mg/L (corresponding to 1.05 mg sodium methyl dithiocarbamate (metam-sodium)/L) and below. The most severe signs were seen at the three highest levels.

Table B.2.9.2-6: Mortality of Bluegill sunfish (*Lepomis macrochirus*), exposed to Vapam (metam-sodium) for 96 hours under static test conditions

Nominal concentration of test item (mg Vapam/L)	Number of fish	Mortality at				Cumulative mortality [%]
		24 hours	48 hours	72 hours	96 hours	
Control	20	0/20	0/20	0/20	0/20	0
0.56	10	0/10	0/10	0/10	0/10	0
1.0	20	0/20	1/20	4/20	4/20	20
1.4	20	0/20	4/20	10/20	15/20	75
1.8	20	0/20	11/20	20/20	20/20	100
3.2	10	0/10	10/10	10/10	10/10	100
10.0	10	10/10	10/10	10/10	10/10	100

Assessment and conclusions:

Results of this investigation indicate that Vapam has a nominal 96 hour LC_{50} of 1.19 mg/L (95 % confidence limits: 1.08 – 1.30 mg/L) in Bluegill sunfish (*Lepomis macrochirus*). This LC_{50} corresponds with to 0.389 mg metam-sodium/L (95 % confidence limits: 0.353 – 0.425 mg metam-sodium/L).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Endpoint:
LC_{50} (*Lepomis macrochirus*, 96 h) = 1.19 mg Vapam/L = 0.389 mg metam-sodium/L (nominal)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Lepomis macrochirus*, 96 h, static) = 1.19 mg Vapam/L = 0.389 mg metam-sodium/L (nominal)
Lainco S.A. notes that no analytical verification of test concentrations was included and hence the study is reported here for supporting information only, on request from the RMS during the completeness check.

Assessment and conclusion by the RMS:
The study is compared with the current guidance.
The validity criteria of OECD Guideline 203 were not all met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (not reported)
- analytical measurement of test concentrations is compulsory (not conducted)

The study shows a lot of shortcomings in comparison to current guidance, however it gives a good indication of the toxicity level of metam-sodium.
LC_{50} (*Lepomis macrochirus*, 96 h, static) = 1.19 mg Vapam/L = 0.389 mg metam-sodium/L (based on nominal concentrations)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/07
Report author:	[redacted]
Report year:	1992
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute Toxicity To The Sheepshead Minnow, *Cyprinodon variegatus*, Under Flow-Through Test Conditions.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-3
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Test temperature: 21.7 – 23.8 °C (recommendation: 23 – 27 °C) Mean Total Length: 3.3 cm (recommendation: 1 – 2 cm) Concentration analysis of test substance was only performed at test initiation and termination. No measurements were made after 48 hours. These deviations were not significant and did not affect the test results.

Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Buckman Laboratories International)

Study Summary:

In a flow-through acute toxicity test, Sheepshead minnows (*Cyprinodon variegatus*) were exposed for 96 hours to 5 nominal concentrations ranging between 7.8 and 60.0 mg Potassium N-methyldithiocarbamate (metam-potassium)/L water and a water control. Actual measured concentrations of metam-potassium were between 6.4 and 66.0 mg metam-potassium/L (77.0 – 110% recovery).
Based upon mean measured concentrations, mortality of Sheepshead minnows exposed for 96 hours to metam-potassium ranged from 0 % at test concentrations ≤ 23 mg metam-potassium/L to 100 % at 66 mg metam-potassium/L. No mortality occurred in the dilution water control. Sub-lethal effects, which included lethargy, dark pigmentation and loss of equilibrium, were observed at concentrations ≥ 10 mg metam-potassium/L.
The 96 hour LC_{50} based upon mean measured concentrations was 30 mg metam-potassium/L with 95 % confidence limits of 23 and 37. The slope of the toxicity curve could not be calculated by binomial probability analysis. The NOEC was 6.4 mg metam-potassium/L (mean measured concentration) based on no mortality or sub-lethal effects occurring at that concentration.

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Age, weight, length, loading:	juveniles, mean weight at start: 1.59 ± 0.41 g (0.98 – 2.63 g), mean length at start: 33 ± 3 mm (27 – 37 mm), fish loading: 0.12 g fish/L
Acclimatisation of the fish:	Acclimatisation period: 10 days, no feeding during the test
Type of test:	Flow-through toxicity test (42 L of test solution with 6.2 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 7.8, 13, 22, 36 and 60 mg a.s./L
Number of animals per group:	20 fish for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 21.7 – 23.8 °C dissolved oxygen: > 6.0 mg/L O_2, > 79 % O_2 saturation pH: 8.0 – 8.4 salinity: 16 – 18 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	The Sheepshead minnow were exposed by groups of 20 for 96 hours to nominal concentrations: 0, 7.8, 13.0, 22.0, 36.0, and 60.0 mg metam-potassium/L in a natural saltwater pumped from a shallow well, carbon treated and then adjusted to a salinity of

approximately 20 ‰ with carbon-treated, aerated laboratory freshwater. Fish loading was 0.12 grams of fish tissue per liter of test water.

Test item analysis: Water samples (3 mL in volume) were collected from the controls and all five test solutions at test initiation and termination to verify actual test concentrations. Water samples were collected from midway in the water column by pipet. Quantitation of metam-potassium was performed by high performance liquid chromatography (HPLC) using a UV-VIS detector and the external standard technique.

Observations: Survival of Sheepshead minnows was monitored daily and any dead removed. Any abnormalities in the behavior or physical appearance of the fish were also noted. Test water quality was monitored daily.

Statistical evaluation: The LC_{50} values were estimated using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for LC_{50} values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0 % and 100 % mortality and the number of concentrations in which mortalities between 0 and 100 % occurred.

Findings:

Analytical results: The diluter functioned properly during the definitive test. The mean measured concentrations during the 96 hour exposure ranged from 6.4 to 66 mg metam-potassium/L and from 77 to 110 % of the nominals.

Mortality: Based upon mean measured concentrations, mortality of Sheepshead minnows exposed for 96 hours to metam-potassium ranged from 0 % at test concentrations ≤ 23 mg metam-potassium/L to 100 % at 66 mg metam-potassium/L. No mortality occurred in the dilution water control. Detailed results of cumulative mortality are presented in the table below.

Behaviour and clinical signs: Sub-lethal effects, which included lethargy, dark pigmentation and loss of equilibrium, were observed at concentrations ≥ 10 mg metam-potassium/L. Detailed results of clinical signs are presented in the table below.

Table B.2.9.2-7: Mortality and clinical signs of Sheepshead minnow (*Cyprinodon variegatus*), exposed to metam-potassium for 96 hours under flow-through test conditions

Nominal concentration of test item (mg a.s./L)	Mean measured concentration of test item (mg a.s./L)	Number of fish	Cumulative number of dead (% mortality)			
			24 hours	48 hours	72 hours	96 hours
Control	-	20	0 (0)	0 (0)	0 (0)	0 (0)
7.8	6.4	20	0 (0)	0 (0)	0 (0)	0 (0)
13	10	20	0 (0)	0 (0)	0 (0)	0[b] (0)
22	22	20	0 (0)	0 (0)	0 (0)	0[c] (0)
36	38	20	0 (0)	0 (0)	6 (30)	19[d] (95)

60	66	20	0[a] (0)	13 (65)	20 (100)	20 (100)

[a] all fish dark; b two fish continuously at water surface; c two fish with complete loss of equilibrium and 18 fish at surface; d surviving fish on its side on the bottom

Assessment and conclusions:

The 96 hour LC_{50} of metam-potassium for Sheepshead minnow (*Cyprinodon variegatus*) based upon mean measured concentrations was 30 mg metam-potassium/L with 95 % confidence limits of 23 and 37 mg metam-potassium/L. The slope of the toxicity curve could not be calculated by binomial probability analysis. The NOEC was 6.4 mg/L (mean measured concentration) based on no mortality or sub-lethal effects occurring at that concentration.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Endpoint:
LC_{50} (*Cyprinodon variegatus*, 96 h) = 30 mg metam-potassium/L (mean measured)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to fish in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: > 79 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/45, for further details).
LC_{50} (*Cyprinodon variegatus*, 96 h, flow-through) = 30 mg metam-potassium/L (based on mean measured concentrations)
NOEC (*Cyprinodon variegatus*, 96 h, flow-through) = 6.4 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/08
Report author:	[redacted]
Report year:	1970b
Report title:	Vapam: Safety Evaluation on *Cyprinodon variegatus* (Broad Killifish).
Report No.:	[redacted]

Document No.:	-
Guidelines followed in study:	None
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Acclimation: 5 days (recommendation: 48 hours settling-in + 7 days acclimation = 9 days) Last feeding: 72 hours before test start (recommendation: 24 – 48 hours before last feeding) Reporting: No detailed information on test material, environmental conditions like pH values or dissolved oxygen concentrations in test medium Test temperature: 15.0 – 17.8 °C (recommendation: 23 – 27 °C) Length of *Cyprinodon variegatus*: 3.7 cm (recommendation: 1 – 2 cm) LC_{50} values were determined after 48 and 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours) No test item analysis was performed (minimum requirement: analysis of the highest and lowest test concentration and a concentration around the expected LC_{50})
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Stauffer Chemical Company)

Study Summary:

In a static acute toxicity test Broad killifish or Sheepshead minnow (*Cyprinodon variegatus*) were exposed to a dilution water control and Vapam nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L (corresponding to 0.183, 0.327, 0.589, 1.05, 1.83 and 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L). Each treatment group consisted of 1 or 2 replicates, each containing 5 fish. No mortality or signs of intoxication were observed in the control group. The derived LC_{50} value for Vapam after 96 hours was 1.3 mg/L (nominal) in Sheepshead minnow. This LC_{50} value corresponds to nominal 0.451 mg metam-sodium/L.

Materials and methods:

Test substance:	Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch no: not reported
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 1.29 g, mean length at start: 3.7 cm, fish loading: 0.43 g fish/L
Acclimatisation of the fish:	7 days acclimatisation, no feeding during the test
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations:

	0 (control), 0.56, 1.0, 1.8, 3.2, 5.6 and 10.0 mg Vapam/L corresponding to 0, 0.18, 0.33, 0.59, 1.05, 1.83 and 3.27 mg a.s./L
Number of animals per group:	5 fish per replicate, 4 replicates for the control and for the treatment groups of 1.8 and 3.2 mg Vapam/L, 2 replicates for the treatment groups of 0.56, 1.0, 5.6 and 10.0 mg Vapam/L
Time of exposure:	96 hours
Test conditions:	temperature: 15 – 17.8 °C dissolved oxygen: not reported pH: not reported salinity: not reported photoperiod: not reported light intensity: not reported
Test procedure:	In a static acute toxicity test Sheepshead minnow (*Cyprinodon variegatus*) were exposed to a dilution water control and Vapam nominal concentrations of 0, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L (corresponding to 0.183, 0.327, 0.589, 1.05, 1.83 and 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L). Therefore, the test material was dissolved in distilled water and aliquots required to attain the desired concentrations were pipetted to jars containing 15 liters. Each treatment group consisted of 1 or 2 replicates, each containing 5 fish.
Test item analysis:	No test item analysis was performed.
Observations:	Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. An LC_{50} was determined at 48 and 96 hours and refers to nominal concentrations.
Statistical evaluation:	Data were plotted graphically and indicated LC_{50} values.

Findings:

Analytical results:	No test item analysis was performed.
Mortality:	No mortalities occurred in the control group or at the treatment levels of 0.56 and 1.0 mg Vapam/L after 96 hours of exposure. Mortality was 100 % at the treatment levels of 1.8, 3.2, 5.6 and 10.0 mg Vapam/L after 96 hours of exposure.
Behaviour and clinical signs:	First signs of intoxication were seen after 24 hours at Vapam nominal concentrations of 3.2 and 5.6 mg/L (corresponding to 1.05 and 1.83 mg metam-sodium/L) and consisted of fish swimming near the surface accompanied by a slight loss of equilibrium and side-swimming. These signs appeared within 24 hours after Vapam exposure and at 1.8 mg/L (0.589 mg metam-sodium/L) after 72 hours exposure. At Vapam nominal concentrations of 10 mg/L (3.27 mg metam-sodium/L), death ensued almost immediately after introduction of the test material.

Table B.2.9.2-8: Mortality of Sheepshead minnow (*Cyprinodon variegatus*), exposed to Vapam (metam-sodium) for 96 hours under static test conditions

Nominal concentration of test item (mg Vapam/L)	Number of fish	Mortality at				Cumulative mortality [%]
		24 hours	48 hours	72 hours	96 hours	

Control	20	0/20	0/20	0/20	0/20	0
0.56	10	0/10	0/10	0/10	0/10	0
1.0	10	0/10	0/10	0/10	0/10	0
1.8	20	0/20	18/20	20/20	20/20	100
3.2	20	5/20	20/20	20/20	20/20	100
5.6	10	8/10	10/10	10/10	10/10	100
10.0	10	10/10	10/10	10/10	10/10	100

Assessment and conclusions:

Results of this investigation indicate that Vapam has a 96 hour LC_{50} of 1.3 mg/L (nominal) in Sheepshead minnow (*Cyprinodon variegatus*). This LC_{50} value corresponds to 0.425 mg metam-sodium/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable as supplementary data. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoint:

LC_{50} (*Cyprinodon variegatus*, 96 h) = 1.3 mg Vapam/L = 0.425 mg metam-sodium/L (nominal)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is mentioned in the dossier of the applicant Lainco.

EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :

LC_{50} (*Cyprinodon variegatus*, 96 h, static) = 1.3 mg Vapam/L = 0.425 mg metam-sodium/L (nominal)

Lainco S.A. notes that no analytical verification of test concentrations was included and hence the study is reported here for supporting information only, on request from the RMS during the completeness check.

Assessment and conclusion by the RMS:

The study is compared with the current guidance.

The validity criteria of OECD Guideline 203 were not all met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (not reported)
- analytical measurement of test concentrations is compulsory (not conducted)

The study shows a lot of shortcomings in comparison to current guidance, however it gives a good indication of the toxicity level of metam-sodium.

LC_{50} (*Cyprinodon variegatus*, 96 h, static) = 1.3 mg Vapam/L = 0.425 mg metam-sodium/L (based on nominal concentrations)

Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.1/09
Report author:	[redacted]
Report year:	1970c
Report title:	Vapam: Safety Evaluation on Striped Majatis.
Report No.:	[redacted]
Document No.:	-

Guidelines followed in study:	None
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Acclimation: 7 days (recommendation: 48 hours settling-in + 7 days acclimation = 9 days) Last feeding: 72 hours before test start (recommendation: 24 – 48 hours before last feeding) Reporting: No detailed information on test material, test fish (scientific name) and environmental conditions like pH values or dissolved oxygen concentrations LC_{50} values were determined after 48 and 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours) No test item analysis was performed (minimum requirement: analysis of the highest and lowest test concentration and a concentration around the expected LC_{50})
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Stauffer Chemical Company)

Study Summary:

In a static acute toxicity test Striped majatis were exposed to a dilution water control and Vapam nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6 and 10.0 mg/L, corresponding to 0.183, 0.327, 0.589, 1.05, 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L. Each treatment group consisted of 2 to 4 replicates, each containing 5 fish. At a Vapam concentration of 10 mg/L death ensued almost immediately after introduction of the test material. The graphically plotted data indicated nominal LC_{50} values after 48 hours and 96 hours as 2.0 and 1.5 mg/L, respectively. These LC_{50} values correspond to 0.654 and 0.491 mg metam-sodium/L after 48 hours and 96 hours, respectively.

Materials and methods:

Test substance:	Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch no: not reported
Test species:	Striped majatis
Age, weight, length, loading:	Age: not reported, mean weight at start: 1.07 g, mean length at start: 4.4 cm, fish loading: 0.36 g fish/L
Acclimatisation of the fish:	Minimum 7 days acclimatisation, no feeding during the test
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.56, 1.0, 1.8, 3.2, 5.6 and 10.0 mg Vapam/L corresponding to 0, 0.18, 0.33, 0.59, 1.05, 1.83 and 3.27 mg a.s./L

Number of animals per group:	5 fish per replicate, 4 replicates for the control and for the treatment groups of 1.8, 3.2 and 5.6 mg Vapam/L, 2 replicates for the treatment groups of 0.56, 1.0 and 10.0 mg Vapam/L
Time of exposure:	96 hours
Test conditions:	temperature: 16.7 – 18.9 °C dissolved oxygen: not reported pH: not reported salinity: not reported photoperiod: not reported light intensity: not reported
Test procedure:	In a static acute toxicity test Striped majatis were exposed to a dilution water control and Vapam nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6 and 10.0 mg/L, corresponding to 0.183, 0.327, 0.589, 1.05, 3.27 mg sodium methyl dithiocarbamate (metam-sodium)/L. Therefore, the test material was dissolved in distilled water and aliquots required to attain the desired concentrations were pipetted to jars containing 15 liters. Each treatment group consisted of 2 or 4 replicates, each containing 5 fish.
Test item analysis:	No test item analysis was performed.
Observations:	Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. An LC_{50} was determined at 48 and 96 hours and refers to nominal concentrations.
Statistical evaluation:	Data were plotted graphically and indicated LC_{50} values.

Findings:

Analytical results:	No test item analysis was performed.
Mortality:	No mortalities occurred in the control group or at the treatment levels of 0.56 and 1.0 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 1.8, 3.2, 5.6 and 10.0 mg Vapam/L after 96 hours of exposure was 85%, 100 %, 100 % and 100%, respectively.
Behaviour and clinical signs:	Signs of intoxication were seen after 24 hours at 5.6, 3.2 mg/L and consisted of fish swimming near the surface accompanied by a slight loss of equilibrium and side-swimming. After 72 hours the symptoms were additionally observed at a concentration of 1.8 mg/L. At a Vapam nominal concentration of 10 mg/L death ensued almost immediately after introduction of the test material.

Table B.2.9.2-9: Mortality of Striped majatis, exposed to Vapam (metam-sodium) for 96 hours under static test conditions

Nominal concentration of test item (mg Vapam/L)	Number of fish	Mortality at				Cumulative mortality [%]
		24 hours	48 hours	72 hours	96 hours	
Control	20	0/20	0/20	0/20	0/20	0
0.56	10	0/10	0/10	0/10	0/10	0
1.0	10	0/10	0/10	0/10	0/10	0
1.8	20	0/20	15/20	16/20	17/20	85

3.2	20	8/20	15/20	20/20	20/20	100
5.6	20	13/20	20/20	20/20	20/20	100
10.0	10	10/10	10/10	10/10	10/10	100

Assessment and conclusions:

Results of this investigation indicate that Vapam has a nominal based 96 hour LC_{50} of 1.5 mg/L in Striped majatis fish. This LC_{50} value corresponds to 0.491 mg metam-sodium/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.
Endpoint:
LC_{50} (*Striped majatis*, 96 h) = 1.5 mg Vapam/L = 0.491 mg metam-sodium/L (nominal)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Striped majatis*, 96 h, static) = 1.5 mg Vapam/L = 0.491 mg metam-sodium/L (nominal)
Lainco S.A. notes that no analytical verification of test concentrations was included and hence the study is reported here for supporting information only, on request from the RMS during the completeness check.

Assessment and conclusion by the RMS:
The study is compared with the current guidance.
The validity criteria of OECD Guideline 203 were not all met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (not reported)
- analytical measurement of test concentrations is compulsory (not conducted)

The study shows a lot of shortcomings in comparison to current guidance, however it gives a good indication of the toxicity level of metam-sodium.
LC_{50} (*Striped majatis*, 96 h, static) = 1.5 mg Vapam/L = 0.491 mg metam-sodium/L (based on nominal concentrations)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Studies with MITC

Data point:	KCA 8.2.1/10
Report author:	[redacted]
Report year:	2002
Report title:	Methylisothiocyanate (metabolite of BAS 002 N, dazomet) Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss*) in a Semistatic System over 96 hours.
Report No.:	[redacted]

Document No.:	[redacted]
Guidelines followed in study:	US EPA Para. 72-1, 1982 US EPA-SEP (Standard Evaluation Procedure) No. 540/9-85-006, 1985 92/69/EEC, Annex V, C1 OECD 203, 1992
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

In a semi-static acute toxicity test, Rainbow trouts (*Oncorhynchus mykiss*) were exposed for 96 hours to 5 nominal concentrations of Methylisothiocyanate (MITC, a metabolite of Dazomet (BAS 002 N)) ranging between 0.05 and 1.0 mg MITC/L water and a water control. Actual measured concentrations of MITC were between 0.032 and 0.90 mg MITC/L (63.9 – 89.8 % recovery).
Mortality of rainbow trout exposed for 96 hours to MITC ranged from 0 % at mean measured concentrations of 0.05 mg MITC/L to 100 % at ≥ 0.22 mg MITC/L. Mortality in the dilution water control was 0 %.
The 96 hour LC_{50} value for Rainbow trout (*Oncorhynchus mykiss*) was 0.08 mg MITC/L based on the nominal concentration of the test substance and 0.0531 mg MITC/L based on the mean measured concentrations. A 96 hour NOEC was determined as 0.05 mg MITC/L (nominal concentration), corresponding to the mean measured value of 0.0320 mg MITC/L.

Materials and methods:

Test substance:	Methylisothiocyanate (MITC), batch no: 408208/1 61100, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	Approximately 3 months old, mean weight at start: 1.0 g (0.8 – 1.2 g), mean length at start: 4.9 cm (4.8 – 5.1 cm), fish loading: 0.64 g fish/L
Acclimatisation of the fish:	14 days acclimatisation, no feeding during the test
Type of test:	Semi-static toxicity test (medium renewal every 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0.05, 0.1, 0.22, 0.5 and 1.0 mg MITC/L
Number of animals per group:	7 fish for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 14 ± 1 °C dissolved oxygen: 6.8 – 10.7 mg/L O_2, 65 – 103 % O_2 saturation pH: 8.1 – 8.4 hardness: 250 mg/L $CaCO_3$ alkalinity: not reported photoperiod: 16 hours light and 8 hours dark light intensity: not reported

Test procedure: In a semi-static acute toxicity test Rainbow trout (*Oncorhynchus mykiss*) were exposed by groups of 7 for 96 hours to nominal concentrations: 0, 0.05, 0.1, 0.22, 0.5 and 1.0 mg MITC/L. The fish loading was 0.6 g of fish per liter of test water.

Test item analysis: Samples for analytical determination of the concentration were taken representatively from each concentration group at start and end of the first and the last interval. Water samples were taken from the middle of the test vessel using a glass pipette stabilized with 20 µL HCI (36 %) and were transported to the analytical laboratory in sealed glass ampoules, which were rinsed with test solution before they were filled. Concentration control analyses of methylisothiocyanate (MITC, a metabolite of Dazomet (BAS 002 N)) in Frankenthal tap water at nominal concentrations of 0.05 mg/L - 1 mg/L as well as in blank control samples were performed using HPLC method CP 057. The test substance is quantified by reversed phase HPLC using a Spherisorb ODS II HPLC column with acetonitrile/water as mobile phase. Quantitation is achieved by UV detection of MITC at 248 nm and external calibration using MITC as reference substance.

Observations: The fish were observed for survival and toxic signs (changes appearance, swimming behaviour and behaviour in comparison to the control group) within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure before and after renewal, respectively. Fish were considered dead if there was no visible movement and no reaction after touching. Dead fish were removed from the test vessels. Temperature, oxygen content and pH-value were measured in each test vessel directly after the test organisms were introduced into the fresh prepared test solution and shortly before removal of the test organism after 24 hours for each of the 4 test intervals of the semi-static exposure.

Statistical evaluation: The median lethal concentration (LC_{50}) after 1, 4, 24, 48, 72 and 96 hours based on the nominal concentrations and based on the mean measured concentrations was calculated using probit analysis. If possible the 95 % confidence intervals were given as well. In case that the data obtained were inadequate for the use of statistical methods for LC_{50} calculation, an approximate LC_{50} was calculated. The geometric mean of the LC_0 and LC_{100} was given as LC_{50} in case that no concentration between LC_0 and LC_{100} was obtained.

Findings:

Analytical results: At the start of the intervals, measured concentrations in the range of 64 – 93 % for recovery were determined, at the end of the intervals the measured concentrations were in a range of 37 - 86 %. The values of the lower concentrations have to be considered with care since they were close to the limit of quantitation (approx. 0.05 mg/L). Losses can be explained by the instability and volatility of the test substance.

Mortality: Mortality of Rainbow trout exposed for 96 hours to MITC ranged from 0 % at mean measured concentrations of 0.05 mg MITC/L to 100 % at ≥ 0.22 mg MITC/L. Mortality in the

dilution water control was 0 %. The 96-hour LC_{50} based on measured MITC concentrations was 0.0531 mg MITC/L.

Behaviour and clinical signs: A no-observed-effect concentration (NOEC) for the 96 hours interval was determined as 0.05 mg MITC/L corresponding to the mean measured value of 0.0320 mg MITC/L. Details of mortality and symptoms observed in the test are presented in the following tables.

Table B.2.9.2-10: Mortality of Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 96 hours under semi-static test conditions

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Number of fish	Cumulative mortality (number of dead organisms)					
			1 h	4 h	24 h	48 h	72 h	96 h
Control	<LOD	7	0	0	0	0	0	0
0.05	0.032	7	0	0	0	0	0	0
0.1	0.067	7	0	0	0	1	5	6
0.22	0.18	7	0	0	0	7	7	7
0.5	0.42	7	0	0	7	7	7	7
1.0	0.90	7	0	0	7	7	7	7

Table B.2.9.2-11: Symptoms for Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 96 hours under semi-static test conditions

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Number of fish	Symptoms (number of affected organisms)					
			1 h	4 h	24 h	48 h	72 h	96 h
control	<LOD	7	N	N	N	N	N	N
0.05	0.032	7	N	N	N	N	N	N
0.1	0.067	7	N	N	N	D (3)	D (2)	D (1)
0.22	0.18	7	N	N	N	-	-	-
0.5	0.42	7	N	N	-	-	-	-
1.0	0.90	7	N	N	-	-	-	-

N – no symptoms observed; D – swimming at the bottom; numbers between brackets- number of animals affected

Assessment and conclusions:

The 96 hour LC_{50} value for Rainbow trout (*Oncorhynchus mykiss*) was 0.08 mg MITC/L based on the nominal concentration of the test substance and 0.0531 mg MITC/L based on the mean measured concentrations. A 96 hour NOEC was determined as 0.05 mg MITC/L corresponding to the mean measured value of 0.0320 mg MITC/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable and fit for purpose.

Endpoints:

LC_{50} (*Oncorhynchus mykiss*, 96 h) = 0.0531 mg MITC/L (mean measured)

NOEC (*Oncorhynchus mykiss*, 96 h) = 0.0320 mg MITC/L (mean measured)

Analytical method:

Despite some deviations from the guidance SANCO/3029/99 rev. 4 on analytical validation, the method is assessed to be fit for purpose, and therefore considered acceptable.

Assessment and conclusion by Lainco:

The study is mentioned in the dossier of the applicant Lainco.

EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Oncorhynchus mykiss*, 96 h, semi-static) = 0.0531 mg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:

- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: 65 – 103 % of saturation)
- analytical measurement of test concentrations is compulsory (see Tables above)

Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/47, for further details). There is uncertainty on the reliability of the endpoint.
LC_{50} (*Oncorhynchus mykiss*, 96 h, semi-static) = 0.0531 mg MITC/L (based on mean measured concentrations)
NOEC (*Oncorhynchus mykiss*, 96 h, semi-static) = 0.0320 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

Data point:	KCA 8.2.1/11
Report author:	[redacted]
Report year:	1991a
Report title:	W150 MITC: The Acute Toxicity of MITC Technical to Rainbow Trout, *Oncorhynchus mykiss*, in a Flow Through System.
Report No.:	[redacted]
Document No.:	[redacted]
Guidelines followed in study:	US EPA PP 72-1
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Last feeding: 52 hours before test start (recommendation: 24 – 48 hours before last feeding) Acclimation: 52 hours (recommendation: 48 hours settling-in + 7 days acclimatisation = 9 days) No detailed information on test chemical analysis and results. No information on preparation of stock solutions. LC_{50} value was determined after 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours)
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: AgrEvo USA Company)

Study Summary:

Rainbow trout (*Oncorhynchus mykiss*) was exposed to nominal concentrations of methyl isothiocyanate (MITC) of 32, 54, 90, 150 and 250 µg/L for 96 hours in a flow-through system according to U.S. EPA OPP 72–1. A dilution water control and a solvent control were tested under the same conditions. Acetone was used as a solvent with a maximum concentration of 0.1 mL/L. Each treatment group consisted of two replicates each containing 10 fish. Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. Recovery of MITC was 81, 74, 87, 87 and 84 % of nominal concentrations. After 96 hours, no mortality was observed in the control groups and in MITC concentrations of 26 to 40 µg/L (mean measured). 3 animals died at a mean measured MITC concentration of 78 µg/L and all fish died in the treatment group with MITC concentrations of 131 and 210 µg/L (mean measured). Calculated 96-hour LC_{50}, based on mean measured concentrations, is 94 µg/L with 95 % confidence limits ranging from 78 to 131 µg/L. The NOEC is 40 µg/L.

Materials and methods:

Test substance:	Methylisothiocyanate (MITC), batch no: not reported, chemical purity: 94.9 %
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 0.64 g, mean length at start: 34 mm, fish loading: 0.074 g fish/L/day
Acclimatisation of the fish:	52 hours acclimatisation, no feeding during the test
Type of test:	Flow-through toxicity test (10 L of test solution with 8.6 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control acetone), 32, 54, 90, 150 and 250 µg MITC/L
Number of animals per group:	10 fish per replicate, 2 replicates for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 11.3 – 12.6 °C dissolved oxygen: > 76 % O_2 saturation pH: 6.2 – 6.5 hardness: 44 mg/L $CaCO_3$ alkalinity: not reported photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	Rainbow trout (*Oncorhynchus mykiss*) was exposed to nominal concentrations of methyl isothiocyanate (MITC) of 32, 54, 90, 150 and 250 µg/L and a dilution water control and solvent control for 96 hours in a flow-through system. Nominal concentrations were based on the results of a 48-hour range finding test with MITC concentrations ranging from 32 to 250 µg/L. Significantly increased mortalities only occurred at the highest test concentration of 250 µg/L. Acetone was used as a solvent with a maximum concentration of 0.1 mL/L. The intermittent-flow proportional diluter of the definite test had a flow-rate of 8.6 volumes per day. Each treatment group consisted of two replicates each containing 10 fish. The loading rate in the 12 L aquaria was 0.64 g/L or 0.074 g/L/day.

Test item analysis:	Test samples were collected from each group at 0 and 96 hours after test initiation and analyzed using GC.
Observations:	Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. Temperature was measured constantly in the water bath. At 0, 48 and 96 hours after test initiation, temperature, pH and dissolved oxygen were measured in each test chamber.
Statistical evaluation:	Binomial probability was used to calculate 96-hour LC_{50} and NOEC. Calculated effect concentrations were based on mean measured concentrations.

Findings:

Analytical results:	Recovery of MITC was 81, 74, 87, 87 and 84 % of nominal concentrations. Mean measured MITC concentrations were 26, 40, 78, 131 and 210 µg/L for the exposure groups. No MITC was detected in the control groups.
Mortality:	After 96 hours, no mortality was observed in the control groups. Mortality of Rainbow trout exposed for 96 hours to MITC ranged from 0 % at mean measured concentrations of ≤ 40 µg/L to 100 % at 131 µg/L and 210 µg/L. Detailed results of cumulative mortality are presented in the table below.
Behaviour and clinical signs:	Lethargy, discoloration, reduced/labored breathing, reduced swimming, loss of equilibrium, spinal curvature and peritoneal lump were signs of toxicity noted among fish exposed at the three highest test concentrations of MITC.

Table B.2.9.2-12: Mortality of Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 96 hours under flow-through test conditions

Nominal concentration of MITC (µg/L)	Mean measured concentrations (µg/L)	Number of fish	Mortality at				Cumulative mortality [%]
			24 hours	48 hours	72 hours	96 hours	
Control	< 10	20	0	0	0	0	0
solvent control	< 10	20	0	0	0	0	0
32	26	20	0	0	0	0	0
54	40	20	0	0	0	0	0
90	78	20	0	0	1	3	15
150	131	20	0	10	19	20	100
250	210	20	3	20	20	20	100

Assessment and conclusions:

The 96 hour LC_{50} of MITC to the Rainbow trout (*Oncorhynchus mykiss*) based on mean measured concentrations was 94 µg/L with 95 % confidence limits ranging from 78 to 131 µg/L. The NOEC is 40 µg/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Oncorhynchus mykiss*, 96 h) = 0.094 mg MITC/L (mean measured)
NOEC (*Oncorhynchus mykiss*, 96 h) = 0.040 mg MITC/L (mean measured)

Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Oncorhynchus mykiss*, 96 h, flow-through) = 0.094 mg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: > 76 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.
The analytical method used could not be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and it cannot be considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/77, for further details). There is uncertainty on the reliability of the endpoint.
LC_{50} (*Oncorhynchus mykiss*, 96 h, flow-through) = 0.094 mg MITC/L (based on mean measured concentrations)
NOEC (*Oncorhynchus mykiss*, 96 h, flow-through) = 0.040 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

Data point:	KCA 8.2.1/12
Report author:	[redacted]
Report year:	1991b
Report title:	W149 MITC: The Acute Toxicity of MITC Technical to Bluegill Sunfish, *Lepomis macrochirus*, in a Flow Through System.
Report No.:	[redacted]
Document No.:	[redacted]
Guidelines followed in study:	US EPA PP 72-1
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Last feeding: 75 hours before test start (recommendation: 24 – 48 hours before last feeding) Acclimation: 5 days (recommendation: 48 hours settling-in + 7 days acclimatisation = 9 days) No detailed information on test chemical analysis and results. No information on preparation of stock solutions. LC_{50} value was determined after 96 hours (recommendation: LC_{50} determination after 24, 48, 72 and 96 hours)
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)

GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: AgrEvo USA Company)

Study Summary:

Bluegill sunfish (*Lepomis macrochirus*) was exposed to nominal concentrations of methyl isothiocyanate (MITC) of 64.8, 108, 180, 300 and 500 µg/L for 96 hours in a flow-through system according to U.S. EPA OPP 72–1. A dilution water control and a solvent control were tested under the same conditions. Acetone was used as a solvent with a maximum concentration of 0.1 mL/L. Each treatment group consisted of two replicates each containing 10 fish. Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. Recovery of MITC was 74, 81, 87, 83 and 80 % of nominal concentrations. After 96 hours, no mortality was observed in the control groups. 13 animals died at mean measured MITC concentrations of 157 µg/L and all animals died at higher MITC concentrations. Calculated 96 hour LC_{50}, based on mean measured concentrations, is 142 µg MITC/L with 95 % confidence limits ranging from 88 to 250 µg MITC/L. The NOEC is 88 µg MITC/L.

Materials and methods:

Test substance:	Methylisothiocyanate (MITC), batch no: not reported, chemical purity: 94.9 %
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 0.608 g, mean length at start: 29 mm, fish loading: 0.071 g fish/L/day
Acclimatisation of the fish:	5 days acclimatisation, no feeding during the test
Type of test:	Flow-through toxicity test (10 L of test solution with 8.6 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0, 64.8, 108, 180, 300 and 500 µg MITC/L
Number of animals per group:	10 fish per replicate, 2 replicates for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 21.1 – 22.9 °C dissolved oxygen: > 83 % O_2 saturation pH: 6.3 – 7.1 hardness: 42 mg/L $CaCO_3$ alkalinity: not reported photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	Bluegill sunfish (*Lepomis macrochirus*) was exposed to nominal concentrations of methyl isothiocyanate (MITC) of 64.8, 108, 180, 300 and 500 µg/L for 96 hours in a flow-through system. Nominal concentrations were based on the results of a 72-hour range finding test with MITC concentrations ranging from 32 to 250 µg/L. Significantly increased mortalities only occurred at the highest test concentration of 250 µg/L. Acetone was used as a solvent with a maximum concentration of 0.1 mL/L. The intermittent-flow proportional diluter of the definite

test had a flow-rate of 8.6 volumes per day. Each treatment group consisted of two replicates each containing 10 fish. The loading rate in the 12 -L aquaria was 0.64 g/L or 0.071 g/L/day.

Test item analysis: Test samples were collected from each group at 0 and 96 hours after test initiation and analysed. No information on the method used.

Observations: Observations were made at regular intervals of 24 hours for signs of intoxication and mortality. Temperature was measured constantly in the water bath. At 0, 48 and 96 hours after test initiation, temperature, pH and dissolved oxygen were measured in each test chamber.

Statistical evaluation: Least squared linear regression versus log_{10} dose was used for 96 hour LC_{50} and NOEC calculation. Verification of statistical results was done by Binomial method. Calculated effect concentrations were based on mean measured concentrations.

Findings:

Analytical results: Recovery of MITC was 74, 81, 87, 83 and 80 % of nominal concentrations. Mean measured MITC concentrations were 48, 88, 157, 251 and 398 µg/L for the exposure groups. No MITC was d etected in the control groups.

Mortality: After 96 hours, no mortality was observed in the control groups and in MITC concentrations of 48 and 88 µg/L (mean measured). 13 animals died at mean measured MITC concentrations of 157 µg/L and all animals died at higher MITC concentrations. Detailed results of cumulative mortality are presented in the table below.

Behaviour and clinical signs: Signs of toxicity including sounding, dark pigmentation and lethargy were observed in fish at the three highest treatment levels of 157, 251 and 398 µg MITC/L.

Table B.2.9.2-13: Mortality of Bluegill sunfish (*Lepomis macrochirus*), exposed to MITC for 96 hours under flow-through test conditions

Nominal concentration of MITC (µg/L)	Mean measured concentrations (µg/L)	Number of fish	Mortality at				Cumulative mortality [%]
			24 hours	48 hours	72 hours	96 hours	
Control	< 10	20	0	0	0	0	0
solvent control	< 10	20	0	0	0	0	0
64.8	48	20	0	0	0	0	0
108	88	20	0	0	0	0	0
180	157	20	0	0	1	13	65
300	251	20	0	20	20	20	100
500	398	20	19	20	20	20	100

Assessment and conclusions:

The 96 hours LC_{50} of MITC to the Bluegill sunfish (*Lepomis macrochirus*) based on mean measured concentrations, was 142 µg MITC/L with 95 % confidence limits ranging from 88 to 250 µg MITC/L. The NOEC is 88 µg MITC/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Lepomis macrochirus*, 96 h) = 0.142 mg MITC/L (mean measured)
NOEC (*Lepomis macrochirus*, 96 h) = 0.088 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
LC_{50} (*Lepomis macrochirus*, 96 h, flow-through) = 0.142 mg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: > 83 % of saturation)
- analytical measurement of test concentrations is compulsory (see Table above)

Therefore, this study is still considered acceptable.
The analytical method used could not be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore it cannot be considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/76, for further details). There is uncertainty on the reliability of the endpoint.
LC_{50} (*Lepomis macrochirus*, 96 h, flow-through) = 0.142 mg MITC/L (based on mean measured concentrations)
NOEC (*Lepomis macrochirus*, 96 h, flow-through) = 0.088 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

Data point:	KCA 8.2.1/13
Report author:	[redacted]
Report year:	2012a
Report title:	Methyl Isothiocyanate (MITC): A 96-hour Flow-through Acute Toxicity Test With The Sheepshead Minnow (*Cyprinodon variegatus*).
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	US EPA OPPTS Nb. 850.1075 ASTM Standard E729-96
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): Test temperature: 21.8 – 22.1 °C (recommendation: 23 – 27 °C) Mean Total Length in Trial 1: 2.3 cm (recommendation: 1 – 2 cm) Light intensity: 253 lux (Trial 1) and 220 lux (Trial 2) (recommendation: 540 – 1000 lux)
Previous evaluation:	No, not previously submitted at EU level

	Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

An initial trial (Trial 1) was conducted but resulted in no observed effects at all concentrations, so the test was repeated at higher concentrations (Trial 2) to allow for the calculation of an LC_{50} value. Data from both test trials were evaluated independently and as a whole to determine the acute effects of Methyl Isothiocyanate (MITC) on the Sheepshead minnow (*Cyprinodon variegatus*). In two flow-through acute toxicity tests, Sheepshead minnows were exposed for 96 hours in 2 replicates of 10 animals per treatment consisting of 5 nominal concentrations ranging between 20 and 600 µg MITC/L in water, a water control and a solvent control. Actual mean measured test concentrations for Trial 1 were 17, 26, 39, 60 and 93 µg a.s./L, representing 85, 87, 89, 90 and 93 % of nominal concentrations, respectively, and for Trial 2 mean measured test concentrations were 37, 67, 142, 281 and 510 µg a.s./L, representing 97, 89, 95, 94 and 85 % of nominal concentrations, respectively.

During Trial 1 all Sheepshead minnows in the negative and solvent control groups appeared normal, with no mortalities or overt signs of toxicity observed. All fish in the 17, 26, 39, 60 and 93 µg a.s./L (mean measured) treatment groups also appeared normal throughout the test, with no mortalities or overt signs of toxicity observed. During Trial 2 all Sheepshead minnows in the negative and solvent control groups and in the 37 and 67 µg a.s./L treatment groups appeared normal throughout the test, with no mortalities or overt signs of toxicity observed. Percent mortality in the 142, 281 and 510 µg a.s./L (mean measured) treatment groups at test termination was 100 % in each group. Signs of toxicity observed among fish in the 142 µg a.s./L (mean measured) treatment groups included surfacing and lying on the bottom of the test chamber. The 96 hour LC_{50} value for Trial 1 was > 93 µg a.s./L, the highest concentration tested. The no-mortality concentration and the NOEC for Trial 1 were both 93 µg a.s./L. The 96 hour LC_{50} value for Trial 2 was 98 µg a.s./L, with a 95 % confidence interval of 67 to 142 µg a.s./L. The no-mortality concentration and the NOEC for Trial 2 were both 67 µg a.s./L. When mortality and observation data for both trials were combined, the 96 hour LC_{50} value was 115 µg a.s./L, with a 95 % confidence interval of 93 to 142 µg a.s./L. The no-mortality concentration and the NOEC were both 93 µg a.s./L, the highest concentration from both trials with no-observed effect.

Materials and methods:

Test substance:	Methyl Isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 99.7 % (according to certificate of analysis)
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Age, weight, length, loading:	Juveniles, Trial 1: mean weight at end: 0.21 g (0.14 – 0.29 g), mean length at end: 2.3 cm (2.1 – 2.6 cm), fish loading: 0.14 g fish/L or 0.014 g fish/L/day Trial 2: mean weight at end: 0.07 g (0.04 – 0.13 g), mean length at end: 1.6 cm (1.5 – 1.9 cm), fish loading: 0.05 g fish/L or 0.015 g fish/L/day
Acclimatisation of the fish:	14 days acclimatisation, no feeding during the test
Type of test:	Flow-through toxicity test (10 volume additions of test water in each test chamber per day)

Applied concentrations:	Nominal test concentrations: Trial 1: 0 (dilution water control), 0 (solvent control 0.1 mL/L dimethylformamide), 20, 30, 44, 67 and 100 µg MITC/L Trial 2: 0 (dilution water control), 0 (solvent control 0.1 mL/L dimethylformamide), 38, 75, 150, 300 and 600 µg MITC/L
Number of animals per group:	10 fish per replicate, 2 replicates for the control, for the solvent control and per treatment group
Time of exposure:	96 hours In life dates: Trial 1: June 27th to July 1st 2011 Trial 2: August 8th to 12th 2011
Test conditions:	temperature: Trial 1: 22.1 – 22.2 °C Trial 2: 21.8 – 22.2 °C dissolved oxygen: Trial 1: 6.0 – 7.10 mg/L O_2, ≥ 76 % O_2 saturation Trial 2: 5.5 – 7.30 mg/L O_2, ≥ 70 % O_2 saturation pH: Trial 1: 8.0 – 8.10 Trial 2: 8.00 – 8.10 salinity: Trial 1 and 2: 20 ‰ photoperiod: Trial 1 and 2: 16 hours light and 8 hours dark light intensity: Trial 1: 253 lux Trial 2: 220 lux
Test procedure:	An initial trial (Trial 1) was conducted but resulted in no observed effects at all concentrations, so the test was repeated at higher concentrations to allow for the calculation of an LC_{50} value. Data from both test trials were evaluated independently and as a whole to determine the acute effects of Methyl Isothiocyanate (MITC) on the Sheepshead minnow (*Cyprinodon variegatus*). Sheepshead minnows were exposed in two replicates of 10 animals per treatment for 96 hours to nominal concentrations: Trial 1 - 0 (negative and solvent control), 20.0, 30.0, 44.0, 67.0 and 100 µg MITC/L; Trial 2 - 0 (negative and solvent control), 38.0, 75.0, 150.0, 300.0 and 600 µg MITC/L. Fish loading was 0.14 and 0.05 grams of tissue per liter of test solution during Trial 1 and Trial 2, respectively.
Test item analysis:	Concentrations of MITC in the samples were determined using an Agilent Model 5890 or 6890 Gas Chromatograph equipped with an Agilent Model 5971A or 5973N Mass Selective Detector operated in the Selective Ion Monitoring (SIM) mode. Chromatographic separations were achieved using a Phenomenex ZB-5 column (30 m x 0.25 mm, 0.25 µm film thickness). Five calibration standards of MITC, ranging in concentration from 10.0 to 100 µg a.s./L, were prepared in diethyl ether using a stock solution of MITC in acetonitrile during Trial 1. Five calibration standards of MITC, ranging in concentration from 20.0 to 200 µg a.s./L, were prepared in diethyl ether using a stock solution of MITC in acetonitrile during Trial 2. Calibration standards prepared at the beginning of Trial 1 were analyzed with each sample set. During Trial 2, fresh calibration standards were prepared and analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of MITC in the samples was

	determined by substituting the peak area responses of the samples into the applicable linear regression equation. The limit of quantitation (LOQ) for the analysis of MITC in acidified saltwater during Trial 1 was set at 10.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard analyzed (10.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00). The limit of quantitation (LOQ) for the analysis of MITC in acidified saltwater during Trial 2 was set at 20.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard analyzed (20.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00). The signal-to-noise (S/N) ratios for four injections of the lowest calibration standard were determined from the analyses performed during each trial. The limit of detection (LOD) was calculated for each standard by dividing the standard concentration by the S/N ratio and multiplying the quotient by three. The mean LOD for MITC during Trial 1 was calculated and reported as 0.704 µg a.s./L. The mean LOD for MITC during Trial 2 was calculated and reported as 18.8 µg a.s./L. Stock solution analyses were conducted during Trial 1 at a nominal concentration of 1000000 µg a.s./L. The mean measured concentration for the stock solution samples was 99.4 % of the nominal concentration. Stock solution analyses were also conducted during Trial 2 at a nominal concentration of 6000000 µg a.s./L. The mean measured concentration for the stock solution samples was 98.5 % of the nominal concentration. During Trial 1, samples of acidified saltwater were fortified at 20.0, 40.0 and 100 µg a.s./L using a stock solution of MITC prepared in acetonitrile and were analyzed concurrently with the samples. The measured concentrations for the matrix fortification samples ranged from 96.3 to 102 % of nominal concentrations. During Trial 2, samples of acidified saltwater were fortified at 35.0, 200 and 600 µg a.s./L using a stock solution of MITC prepared in acetonitrile and were analyzed concurrently with the samples. The measured concentrations for the matrix fortification samples ranged from 94.6 to 108 % of nominal concentrations.
Observations:	All organisms were observed periodically to determine the number of mortalities in each treatment group. The numbers of individuals exhibiting signs of toxicity or abnormal behavior were also evaluated. Observations were made approximately 3, 24, 48, 72 and 96 hours after test initiation during Trial 1. Observations were made approximately 4.5, 24, 48, 72 and 96 hours after test initiation during Trial 2. Temperature was measured in each test chamber at the beginning and end of each test trial using a liquid-in-glass thermometer. Temperature also was monitored continuously in one negative control test chamber during each trial using a Fulscope ER/C Recorder, which was verified prior to test initiation with a liquid-in-glass thermometer dissolved oxygen and pH were measured in one replicate test chamber of each treatment and control group at the beginning of the test, at approximately 24-hour intervals during the test, and at the end of the test, with measurements alternating between replicates in each group at each

measurement interval. Dissolved oxygen was measured using a Thermo Orion Model 850Aplus dissolved oxygen meter, and measurements of pH were made using a Thermo Orion Model 525Aplus meter. When 100 % mortality occurred in a test chamber, measurements of temperature, dissolved oxygen and pH were taken in that test chamber and then discontinued. Salinity was measured in the dilution water at the beginning and end of each test trial using a VitalSine refractometer.

Statistical evaluation:

The absence of mortality in any of the MITC treatment groups during Trial 1 of the test precluded the statistical calculation of LC_{50} values at 24, 48, 72 and 96 hours. Therefore, the LC_{50} values for Trial 1 were estimated to be greater than the highest concentration tested. In Trial 2, non-linear interpolation was used to calculate the 24, 48, 72 and 96 hour LC_{50} values and binominal probability was used to calculate the 95 % confidence intervals. Due to the method used to calculate the 96 hour LC_{50} value during Trial 2, the slope of the dose response curve could not be calculated. Mortality and observation data for both trials were combined to calculate a 96 hour LC_{50} value based on the same procedures described above for Trial 2. The no-mortality concentration and NOEC for both trials and the combined date for both trials were determined by visual interpretation of the mortality and observation data.

Findings:

Analytical results:

Trial 1:

Measured concentrations of the stock samples ranged from approximately 99 to 100 % of nominal, indicating that stocks being delivered to the test system were at the targeted nominal concentrations. Measured concentrations in the pretest diluter verification samples ranged from approximately 85 to 93 % of nominal. Measured concentrations of MITC in the test solution samples ranged from approximately 83 to 99 % of nominal. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 17, 26, 39, 60 and 93 µg a.s./L, representing 85, 87, 89, 90 and 93 % of nominal concentrations, respectively.

Trial 2:

Measured concentrations of the stock samples ranged from approximately 98 to 99 % of nominal, indicating that stocks being delivered to the test system were at the targeted nominal concentrations. Measured concentrations of MITC in the pretest diluter verification samples ranged from approximately 83 to 96 % of nominal. Measured concentrations of MITC in the test solution samples ranged from approximately 84 to 106 % of nominal. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 37, 67, 142, 281 and 510 µg a.s./L, representing 97, 89, 95, 94 and 85 % of nominal concentrations, respectively.

Mortality, behaviour and clinical signs:	Trial 1: All Sheepshead minnows in the negative and solvent control groups appeared normal throughout the test, with no mortalities or overt signs of toxicity observed. All fish in the 17, 26, 39, 60 and 93 µg a.s./L treatment groups also appeared normal throughout the test, with no mortalities or overt signs of toxicity observed. Trial 2: All Sheepshead minnows in the negative and solvent control groups and in the 37 and 67 µg a.s./L treatment groups appeared normal throughout the test, with no mortalities or overt signs of toxicity observed. Percent mortality in the 142, 281 and 510 µg a.s./L treatment groups at test termination was 100 % in each group. Signs of toxicity observed among fish in the 142 µg a.s./L treatment groups included surfacing and lying on the bottom of the test chamber. Detailed results of cumulative mortality for both trials are presented in the tables below.

Table B.2.9.2-14: Trial 1: Mortality and symptoms for Sheepshead minnow (*Cyprinodon variegatus*), exposed to MITC for 96 hours under flow-through test conditions

Nominal concentration of test item (µg a.s./L)	Mean measured concentration of test item (µg a.s./L)	Number of fish exposed	Cumulative mortality (number of dead organisms)					
			0 hours		24 hours		48 hours	
			No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]
Control	<LOQ	20	0	20 AN	0	20 AN	0	20 AN
Solvent control	<LOQ	20	0	20 AN	0	20 AN	0	20 AN
20	17	20	0	20 AN	0	20 AN	0	20 AN
30	26	20	0	20 AN	0	20 AN	0	20 AN
44	39	20	0	20 AN	0	20 AN	0	20 AN
67	60	20	0	20 AN	0	20 AN	0	20 AN
100	93	20	0	20 AN	0	20 AN	0	20 AN

[1] *cumulative number of dead fish*

[2] *observations: AN = appear normal*

The limit of quantification (LOQ) was 10.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00)

Nominal concentration of test item (µg a.s./L)	Mean measured concentration of test item (µg a.s./L)	Number of fish exposed	Cumulative mortality (number of dead organisms)				Cumulative mortality (%)
			72 hours		96 hours		
			No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]	
Control	<LOQ	20	0	20 AN	0	20 AN	0
Solvent control	<LOQ	20	0	20 AN	0	20 AN	0
20	17	20	0	20 AN	0	20 AN	0
30	26	20	0	20 AN	0	20 AN	0
44	39	20	0	20 AN	0	20 AN	0
67	60	20	0	20 AN	0	20 AN	0
100	93	20	0	20 AN	0	20 AN	0

[1] *cumulative number of dead fish*

[2] observations: AN = appear normal
The limit of quantification (LOQ) was 10.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00)

Table B.2.9.2-15: Trial 2: Mortality and symptoms for Sheepshead minnow (*Cyprinodon variegatus*), exposed to MITC for 96 hours under flow-through test conditions

Nominal concentration of test item (µg a.s./L)	Mean measured concentration of test item (µg a.s./L)	Number of fish exposed	Cumulative mortality (number of dead organisms)					
			0 hours		24 hours		48 hours	
			No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]
Control	<LOQ	20	0	20 AN	0	20 AN	0	20 AN
Solvent control	<LOQ	20	0	20 AN	0	20 AN	0	20 AN
38	37	20	0	20 AN	0	20 AN	0	20 AN
75	67	20	0	20 AN	0	20 AN	0	20 AN
150	142	20	0	20 AN	0	20 AN	0	20 AN
300	281	20	0	20 AN	0	20 AN	20	-
600	510	20	0	20 AN	20	-	20	-

[1] cumulative number of dead fish
[2] observations: AN = appear normal
The limit of quantification (LOQ) was 20.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (20.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00)

Nominal concentration of test item (µg a.s./L)	Mean measured concentration of test item (µg a.s./L)	Number of fish exposed	Cumulative mortality (number of dead organisms)				Cumulative mortality (%)
			72 hours		96 hours		
			No. dead[1]	Obs.[2]	No. dead[1]	Obs.[2]	
Control	<LOQ	20	0	20 AN	0	20 AN	0
Solvent control	<LOQ	20	0	20 AN	0	20 AN	0
38	37	20	0	20 AN	0	20 AN	0
75	67	20	0	20 AN	0	20 AN	0
150	142	20	8	4 AN, 7A, 1R	20	-	100
300	281	20	20	-	20	-	100
600	510	20	20	-	20	-	100

[1] cumulative number of dead fish
[2] observations: AN = appear normal, A = surfacing, R = lying on the bottom of the chamber
The limit of quantification (LOQ) was 20.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (20.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00)

Assessment and conclusions:

The 96 hour LC_{50} value for Trial 1 was > 93 µg a.s./L, the highest concentration tested. The no-mortality concentration and the NOEC for Trial 1 were both 93 µg a.s./L. The 96 hour LC_{50} value for Trial 2 was 98 µg a.s./L, with a 95 % confidence interval of 67 to 142 µg a.s./L. The no-mortality concentration and the NOEC for Trial 2 were both 67 µg a.s./L. When mortality and observation data for both trials were combined, the 96 hour LC_{50} value was 115 µg a.s./L, with a 95 % confidence interval of 93 to 142 µg a.s./L. The no-mortality concentration and the NOEC were both 93 µg a.s./L, the highest concentration from both trials with no-observed effect.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Cyprinodon variegatus*, 96 h) = 115 µg MITC/L (mean measured)
Analytical method:
Despite some minor deviations from the guidance SANCO/3029/99 rev. 4 on analytical validation, the method is assessed to be fit for purpose, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 % in Trial 1 and 2)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: ≥ 76 % of saturation in Trial 1 and ≥ 70 % of saturation in Trial 2)
- analytical measurement of test concentrations is compulsory (see Tables above)

Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/48, for further details).
LC_{50} (*Cyprinodon variegatus*, 96 h, flow-through) = 115 µg MITC/L (based on mean measured concentrations)
NOEC (*Cyprinodon variegatus*, 96 h, flow-through) = 93 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

Data point:	KCA 8.2.1/14
Report author:	[redacted]
Report year:	2019a
Report title:	Methyl isothiocyanate (MITC) - Acute Toxicity to Rainbow trout (*Oncorhynchus mykiss*) in a 96-Hour Test.
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	OECD No. 203 (1992) Commission Regulation (EC) No 440/2008, Part C.1 (2008)
Deviations from current test guideline:	Deviations from current OECD guideline 203 (2019): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities (exception: pre-test for verification of the stability of the test item in ethanol (non-GLP))
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

	(original Sponsor: Kanesho Soil Treatment, letter of co-ownership by Taminco is included, study may be used by Taminco without restriction in Europe only)

Study Summary:

The acute toxicity of the test item Methyl isothiocyanate (MITC) to rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour test.
The test was run under flow-through conditions for a test period of 4 days. At the start of the test, seven Rainbow trout (*Oncorhynchus mykiss*) each were exposed to test media containing the test item at different concentrations. The fish were observed for visible abnormalities and mortality.
For the dosage of the test item in test water, the solvent ethanol was used. The nominal concentrations of the test item of 10, 22, 46, 100 and 220 µg/L were tested. Additionally, a control and a solvent control group were tested in parallel.
In the test medium samples from the analysed test media of nominal 22 to 220 µg/L the measured concentrations of Methyl isothiocyanate (MITC) were in the range of 77 – 92 % of the nominal values. The losses observed were attributed to specific properties of the test item, i.e. the high volatility.
Up to the test item concentration of 20 µg/L (mean measured), no toxic effects were observed in the fish until test end. At the next higher test item concentrations of 35 and 86 µg/L, visible abnormalities were observed starting from day 2 and at the end of the test one and two fish were found to be dead at these test concentrations. At the highest test item concentration of 186 µg/L visible abnormalities were observed at day 1 and after 2 days of exposure all fish were dead.
The 96 hour LC_{50} was 90 µg MITC/L. The 96 hour NOEC was 20 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	Age: not reported, mean weight at start: 0.87 ± 0.08 g, mean length at start: 4.7 ± 0.12 cm, fish loading: 0.5 g fish wet weight/L medium
Acclimatisation of the fish:	1 week acclimatisation, no feeding during the test
Type of test:	Flow-through toxicity test The frequency of test medium preparation cycles was 10 minutes, i.e. 6 cycles per hour. In total, 144 L of test medium per day were used for each treatment. As 12 L flow-through aquaria were used, this test medium flow resulted in a 12-fold theoretical test medium exchange rate per day.
Applied concentrations:	Nominal test concentrations: 0 (dilution water control), 0 (solvent control 60 µL/L acetone), 10, 22, 46, 100 and 220 µg MITC/L
Number of animals per group:	7 fish for the control, for the solvent control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 13 - 14 °C dissolved oxygen: 9.0 – 9.3 mg/L O_2 pH: 7.2 – 7.4 total hardness: 125 mg/L $CaCO_3$ alkalinity: 0.4 mmol/L photoperiod: 16 hours light and 8 hours dark light intensity: 10 – 12 µE s^{-1} m^{-2}

Test procedure:	For determination of the appropriate test item concentrations for the main test, a range-finding test was performed. The test was performed under the same experimental conditions (flow-through) as the main test. For each treatment, three fish were used. Based on the results of the range finding test, the following nominal concentrations of the test item were tested in the main test: 10, 22, 46, 100 and 220 µg/L. Additionally, a control and a solvent control (60 µL/L acetone) were tested in parallel (test water without test item). The test was run under flow-through conditions. For continuous dosing of the test item into test water a computer-controlled automated flow-through dosing system was used. The whole dosing system consisted of individual dosing units for each treatment. For the dosing of the test item into test water, concentrated application solutions were prepared for each test item concentration. At the start of the test, seven fish were introduced into each aquarium in a random order. The loading rate of the fish was 0.5 g fish wet weight per liter medium.
Test item analysis:	For measurement of the actual concentrations of the test item, duplicate 500 mL samples taken from all test media with surviving fish and from the solvent control at the start of the test and each day thereafter. Immediately after sampling, all samples were frozen (at -20 ± 5 °C). The concentrations of the test item in the test media and the solvent control were analysed in one of the duplicate samples from the test item concentrations of 22 to 220 µg/L from the start of the test and from day 2 and day 4 (with the exception of the highest test concentration of 220 µg/L where no samples were available from day 4 as all fish were dead already at day 2). Additionally, for day 1 and day 3, one of the duplicate samples were analysed from the test concentrations of nominal 46 and 100 µg/L. The samples from the test concentration of nominal 10 µg/L were not analyses as this test concentration was below the NOEC determined in this test and therefore not relevant for the interpretation of the biological results. The analytical method used is gas chromatography with mass spectrometric detection (GC/MS).
Observations:	The test fish were observed for mortality and visible abnormalities after approximately 2, 24, 48, 72 and 96 hours test duration. The water temperature, pH values and oxygen concentrations were measured for each treatment with surviving fish at the start of the test and each day thereafter. At the same dates the appearance of the test media was recorded. Additionally, the water temperature was recorded with a data logger.
Statistical evaluation:	The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the Raw Data. The 24 to 72 hour LC_{50} at the observation times and the 95 % confidence limits were calculated as far as possible by the Trimmed Spearman-Karber procedure. Statistical analysis was performed using ToxRat Professional®.

Findings:

Analytical results:	No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the test period of 96 hours. In the test medium samples from the analysed test media of nominal 22 to 220 µg/L, the measured concentrations of Methyl isothiocyanate (MITC) were in the range of 77 – 92 % of the nominal values. The losses observed were attributed to specific properties of the test item, i.e. the high volatility. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test item concentration. The biological results are presented based on the mean measured test item concentrations.
Mortality, behaviour and clinical signs:	Up to the test item concentration of 20 µg/L (mean measured), no toxic effects were observed in the fish until test end. At the next higher test item concentrations of 35 and 86 µg/L, visible abnormalities were observed starting from day 2 and at the end of the test one and two fish were found to be dead at these test concentrations. At the highest test item concentration of 186 µg/L visible abnormalities were observed at day 1 and after 2 days of exposure all fish were dead. The biological endpoints (based on mean measured concentrations) were as follows: 96 hour LC_{50} = 90 µg/L (95 % confidence limits: 64 – 127 µg/L) 96 hour LC_0 = 20 µg/L 96 hour LC_{100} = 186 µg/L 96 hour NOEC = 20 µg/L 96 hour LOEC = 35 µg/L

Table B.2.9.2-16: Mortality and symptoms for Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 96 hours under flow-through test conditions

Nominal test item concentration (µg/L)	**Mean measured test item concentration (µg/L)**	**Number of abnormal fish / Number of dead fish**				
		2 hours	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	Control	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Solvent control	Solvent control	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
10	n.a.	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
22	20	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
46	35	0 / 0	0 / 0	3 / 0 UV, VF	3 / 0 UV, VF	6 / 1 UV, VF
100	86	0 / 0	0 / 0	3 / 0 UV, VF	7 / 0 UV, VF	5 / 2 UV, VF, TS, AP
220	186	0 / 0	7 / 0 UV, VF	0 / 7	--	--
LC_{50} (µg/L)		> 186	> 186	127	127	90
95 % confidence interval		n.d.	n.d.	n.d.	n.d.	64 - 127

n.a. = not analysed since below the NOEC determined in this test, n.d. = not determined
-- = all fish dead

Abbreviations of visible abnormalities observed in the test fish during the study
UV : atypical behaviour, i.e. fish mainly at the bottom of the aquarium
SA : mucus secretion
SV : heavy breathing (clearly visible increased breathing frequency)
VF : changed body color
AP: apathy
KR : convulsions
TS : tumbling during swimming
AK : strongly extended gills
BA : distended abdomen
GA : exophthalmus
SR : fish lying on side or back on the bottom

Assessment and conclusions:

The test item Methyl isothiocyanate (MITC) had acute toxic effects to Rainbow trout (*Oncorhynchus mykiss*) in a 96 hour flow-through test.
The 96 hour LC_{50} was calculated to be 90 µg/L with 95 % confidence limits of 64 and 127 µg/L (based on mean measured concentrations).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Oncorhynchus mykiss*, 96 h) = 90 µg MITC/L (mean measured)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 203 were met:
- the mortality in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration should be ≥ 60 % of the air saturation value (measured: ≥ 60 % of saturation)
- analytical measurement of test concentrations is compulsory (see Tables above)

Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/70 for further details).
LC_{50} (*Oncorhynchus mykiss*, 96 h, flow-through) = 90 µg MITC/L (based on mean measured concentrations)
NOEC (*Oncorhynchus mykiss*, 96 h, flow-through) = 20 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. 5 acute (short-term) toxicity studies with aquatic invertebrates are available for metam (performed with either metam-sodium or metam-potassium), and 17 such studies are available for MITC.

Studies with metam

Data point:	KCA 8.2.4.1/01
Report author:	[redacted]
Report year:	1985
Report title:	Determination of the Acute Toxicity of BAS 005 00 N METAM SODIUM 510 g/L to the waterflea *Daphnia magna* Straus.
Report No.:	85/0497
Document No.:	1/0057/2/85-279/85
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-2
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): No analytical measurements of test substance (recommendation: as a minimum measurements at highest and lowest test concentration)
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF Corporation Chemicals Division)

Study Summary:

In this study the acute toxicity of BAS 005 00 N Metam Fluid 510 g/L BASF to the waterflea *Daphnia magna* has been determined. The investigation was carried out following the draft proposal of the German standard method DIN 38 412 Part 11.
The concentration of the substance in the stock solution was 62.5 mg/L. A serial dilution in dilution water was prepared. To each test vessel 288 ml of the respective dilution were added.
At the beginning and after 24 and 48 hours *Daphnia magna*'s inability to swim is recorded. $EC_{50,}$ EC_0 and EC_{100} after 24 and 48 hours were determined. The EC_{50} based on nominal concentrations of BAS 005 00 N Metam Fluid 510 g/L BASF to *Daphnia magna* was after 48 h: EC_{50} (48 h) = 2.34 mg/L.

Materials and methods:

Test substance:	Metam Fluid 510 g/L (BAS 005 00 N), formulation containing 42.2 % metam-sodium, batch no: not reported
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	6 – 24 hours old
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.122, 0.244, 0.488, 0.976, 1.95, 3.9, 7.81, 15.6, 31.2 and 62.5 mg Metam Fluid/L

Number of organisms per group:	5 daphnids per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: September 3rd to 5th 1985
Test conditions:	temperature: 20 °C dissolved oxygen: 8.29 – 8.6 mg/L O_2, 90 – 93 % O_2 saturation pH: 7.99 – 8.89 (at 0 hours), 7.84 – 7.91 (at 48 hours) total hardness: 2.68 mmol/L alkalinity: not reported lighting: none, except for determination of the ability to swim
Test procedure:	Daphnids were assigned to the test groups listed in the table below. They were exposed by groups of 5 in 4 replicates for 48 hours to nominal concentrations of 0.0, 0.122, 0.244, 0.488, 0.976, 1.95, 3.9, 7.81, 15.6, 31.2 and 62.5 mg BAS 005 00 N Metam Fluid 510 g/L BASF/L in reconstituted water. Tap water was used as the test water, purified by charcoal to remove chlorine and filtered through a 6 µm filter. The buffering capacity of the carbonic acid system was reduced by addition of sulfuric acid. To reduce the total hardness, deionized water was added.
Test item analysis:	No test item analysis was performed.
Observations:	At the beginning and after 24 and 48 hours *Daphnia magna*'s inability to swim is recorded. Those animals which are unable to swim after gentle agitation of the test vessels are regarded as unable to swim.
Statistical evaluation:	Determination of the $EC_{50,}$ EC_0 and EC_{100} after 24 and 48 hours. For determination of the $EC_{50,}$ after 48 hours the moving average method was used. The EC_0 is the highest concentration tested with an effect ≤ 10 %. The EC_{100} is the lowest concentration tested with an effect of 100 %. These values are based on nominal concentrations.

Findings:

Analytical results:	No test item analysis was performed.
Immobilisation:	No immobility was observed in the control group and up to the treatment level of 0.976 mg Metam Fluid/L after 48 hours of exposure. Immobility at the treatment levels of 1.95, 3.9, 7.81, 15.6, 31.2 and 62.5 mg Metam Fluid/L after 48 hours of exposure was 55 %, 70 %, 100 %, 100 %, 100 % and 100 %, respectively. The 24 hour EC_{50} could not be determined. The EC_0 and EC_{100} at 24 hours were determined to be 31.2 mg/L and > 62.5 mg/L, respectively. The 48 hour EC_{50} was determined to be 2.34 mg/L (95 % confidence limits: 1.83 – 2.85 mg/L). The EC_0 and EC_{100} at 48 hours were determined to be 0.976 mg/L and 7.81 mg/L, respectively.

Table B.2.9.2.2-17: Immobility of *Daphnia magna*, exposed to metam-sodium for 48 hours under static test conditions

Nominal concentration of test item (mg BAS 005 00 N Metam Fluid 510 g/L BASF/L)	Daphnids able to swim after		
	0 hours	24 hours	48 hours

control	20	20	20
0.122	20	20	20
0.244	20	20	20
0.488	20	20	20
0.976	20	20	20
1.95	20	20	9
3.9	20	20	6
7.81	20	20	0
15.6	20	18	0
31.2	20	20	0
62.5	20	5	0

Assessment and conclusions:

The EC_{50} based on nominal concentrations of BAS 005 00 N Metam Fluid 510 g/L BASF to *Daphnia magna* was after 48 hours:

EC_{50} = 2.34 mg test item/L
CI 95% = 1.83 – 2.85 mg test item /L

The highest test concentration without effect was after 48 hours:

EC_0 = 0.976 mg test item /L

The lowest tested concentration with 100 % effect was after 48 hours:

EC_{100} = 7.81 mg test item /L

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Endpoints:
EC_{50} (*Daphnia magna*, 48 h) = 2.34 mg Metam Fluid/L = 0.99 mg metam-sodium/L (nominal)
EC_0 (*Daphnia magna*, 48 h) = 0.976 mg Metam Fluid/L = 0.41 mg metam-sodium/L (nominal)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
EC_{50} (*Daphnia magna*, 48 h, static) = 2.34 mg Metam Fluid/L = 0.99 mg metam-sodium/L (nominal)
Lainco S.A. notes that no analytical verification of test concentrations was included and hence the study is reported here for supporting information only, on request from RMS during the completeness check.

Assessment and conclusion by the RMS:
The study is compared with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)

- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.29 – 8.6 mg/L O_2)

However, analytical measurement of test concentrations was not performed, as recommended by OECD Guideline 202 when reporting the data. The study shows shortcomings in comparison to current guidance, however it gives a good indication of the toxicity level of metam-sodium.
EC_{50} (*Daphnia magna*, 48 h, static) = 2.34 mg Metam Fluid/L = 0.99 mg metam-sodium/L (based on nominal concentrations)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.4.1/02
Report author:	[redacted]
Report year:	1993
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute Toxicity To The Water Flea, *Daphnia magna*, Under Flow-Through Test Conditions.
Report No.:	J9110001b
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-2
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Metam Sodium Task Force)

Study Summary:

A flow-through freshwater toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to determine the acute toxicity of potassium N-methyldithiocarbamate (metam-potassium) to the immobilization (i.e., no movement except for minor activity of appendages).
A flow-through range-finding test was conducted using 10 water fleas per test concentration (2.3, 3.9, 6.5, 10.8, 18.0 and 30.0 mg/L) prior to performing the definitive test. After 48 hours of exposure, mortality was ≤ 10 % at concentrations ≤ 10.8 mg/L, 80 % at 18.0 mg/L, and 100 % at 30 mg/L. Based upon the results of this range-finding test, the nominal metam-potassium concentrations selected for the definitive test were 1.4, 2.3, 3.9, 6.5, 10.8 and 18 mg/L.
The definitive exposure was conducted under flow-through conditions in a modified proportional vacuum-siphon diluter system. The diluter system was constructed of glass, silicone adhesive, and silicone tubing. The test system was volumetrically calibrated to provide a test concentration series with a 60 % dilution. Neat metam-potassium was injected directly into the chemical mixing chamber at a rate of 13.18 µL during each diluter cycle which provided a high nominal test concentration of 18.0 mg/L. This test solution was proportionally diluted in the test system to additionally provide the five lower test concentrations (i.e. 10.8, 6.5, 3.9, 2.3 and 1.4 mg/L). A dilution water control was maintained concurrently with the six test solutions.

Results of the test are expressed as a 48-hour median effective concentration (EC_{50}), the concentration of metam-potassium calculated to results in immobilization or death to 50 percent of the test population at the specified time.

EC_{50} (24 h) < 19.1 mg metam-potassium/L
EC_{50} (48 h) = 6.34 mg metam-potassium/L

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	Neonates, < 24 hours old
Type of test:	Flow-through toxicity test (19 volume renewals per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 1.4, 2.3, 3.9, 6.5, 10.8 and 18.0 mg a.s./L
Number of organisms per group:	10 daphnids per replicate, 2 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: August 6th to 8th 1993
Test conditions:	temperature: 20.0 – 20.3 °C dissolved oxygen: 6.7 – 7.7 mg/L O_2, 74 – 85 % O_2 saturation, ≥ 7.0 mg/L O_2, ≥ 77 % O_2 saturation pH: 6.7 – 7.0 total hardness: 56 mg/L $CaCO_3$ alkalinity: 6 – 9 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 282 – 310 lux
Test procedure:	A flow-through range-finding test was conducted using 10 water fleas per test concentration (2.3, 3.9, 6.5, 10.8, 18.0 and 30.0 mg/L) prior to performing the definitive test. After 48 hours of exposure, mortality was ≤ 10% at concentrations ≤ 10.8 mg/L, 80 % at 18.0 mg/L, and 100 % at 30 mg/L. Based upon the results of this range-finding test, daphnids were exposed by groups of 10 in 2 replicates for 48 hours to nominal concentrations of 0, 1.4, 2.3, 3.9, 6.5, 10.8 and 18 mg/L.
Test item analysis:	Test media was analysed for determination of metam-potassium via HPLC at 0 hour (fresh test solution) and after 48 hours (aged test solutions) for control and all tested concentrations.
Observations:	Survival of *Daphnia magna* was monitored daily and any dead or immobilised animals were removed. Any abnormalities in the behavior or physical appearance of the daphnids were also noted.
Statistical evaluation:	Based on the results of the test, the 24 and 48 hour EC_{50} values and their 95 % confidence limits were calculated. The EC_{50} values were estimated by a computer program using the following statistical methods: moving average angle, probit, logit, and non-linear interpolation. Confidence limits for EC_{50} values determined by non-linear interpolation were calculated by binominal probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0

% and 100 % mortality and the number of concentrations in which mortalities between 0 % and 100 % occurred.

Findings:

Analytical results: Measured metam-potassium concentrations closely approximated nominal concentrations throughout the 48 hour exposure. Mean measured metam-potassium concentrations ranged from 1.34 to 19.1 mg a.s./L and from 96 to 120 % of nominal.

Immobilisation: Mortality (or immobilisation) of daphnids exposed for 48 hours to metam-potassium ranged from 0 % at test concentrations ≤ 4.68 mg a.s./L to 80 % at 19.1 mg a.s./L. Lethargy was observed on one or more animals after 48 hours of exposure at test concentrations ≥ 2.44 mg a.s./L. The 48 hour EC_{50} was 6.34 mg a.s./L with 95 % confidence limits of 4.67 and 11.2 mg a.s./L. The no-observed-effect concentration (NOEC) was 1.34 mg a.s./L based on a lack of mortality and sublethal effects at this test concentration.

The 24 and 48 hour EC_{50} values were determined to be < 19.1 mg a.s./L and 6.34 mg a.s./L (95 % confidential limits at 48 hours: 4.67 and 11.2 mg a.s./L), respectively.

Table B.2.9.2.2-18: Immobility of *Daphnia magna*, exposed to metam-potassium for 48 hours under flow-through test conditions

Nominal concentrations (mg/L)	Mean measured concentrations (mg/L)	No. of daphnids	Cumulative number affected (percent)	
			24 hours	48 hours
0	0	2*10	0 (0)	0 (0)
1.4	1.34	2*10	0 (0)	0 (0)
2.3	2.44	2*10	0 (0)	0 (0)[d]
3.9	4.68	2*10	0 (0)	0 (0)[e]
6.5	6.63	2*10	0 (0)[a]	12 (60)[f]
10.8	11.2	2*10	0 (0)[b]	15 (75)[a]
18.0	19.1	2*10	3 (15)[c]	16 (80)[a]

[a] Four daphnids lethargic, [b] Two daphnids lethargic, [c] Nine daphnids lethargic, [d] One daphnids lethargic, [e] Ten daphnids lethargic, [f] Eight daphnids lethargic

Assessment and conclusions:

In the conditions of the test, the 48 hour EC_{50} of metam-potassium to *Daphnia magna* was determined to be 6.34 mg a.s./L with corresponding 95 % confidential limits of 4.67 and 11.2 mg metam-potassium/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable as supplementary data.

Endpoints:

EC_{50} (*Daphnia magna*, 48 h) = 6.34 mg metam-potassium/L (mean measured)

NOEC (*Daphnia magna*, 48 h) = 1.34 mg metam-potassium/L (mean measured)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to fish in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: ≥ 7.0 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B..1.2.6 – KCA 4.1.2/44, for further details).
EC_{50} (*Daphnia magna*, 48 h, flow-through) = 6.34 mg metam-potassium/L (based on mean measured concentrations)
NOEC (*Daphnia magna*, 48 h, flow-through) = 1.34 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.4.1/03
Report author:	[redacted]
Report year:	2013
Report title:	Metam sodium (technical grade): Acute toxicity to *Daphnia magna*.
Report No.:	PQB0016
Document No.:	-
Guidelines followed in study:	EC Methods for Determination of Ecotoxicity, Annex to Commission Regulation (EC) No 440/2008 Part C, Method 2 "*Daphnia* sp. Acute Immobilisation Test" OECD Guideline for Testing of Chemicals No. 202, "*Daphnia* Acute Immobilisation Test" (2004)
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): None
Previous evaluation:	No, not previously submitted Accepted to support Lainco S.A.'s Step 1 application (2015, RMS Belgium)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	This study is in line with the current guidelines and therefore considered acceptable. However, as the test item is metam-sodium, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Study owner:	Lainco S.A. (letter of co-ownership by Taminco is included, study may be used by Taminco without restriction for registration purposes)

Study Summary:

The acute toxicity of metam-sodium (technical grade) to *Daphnia magna* was assessed under sealed, semi-static (24 hour renewal period) exposure conditions over a period of 48 hours in accordance with OECD test guideline No. 202 (2004).
Following a range-finding test, in the definitive test juvenile daphnids (< 24 hours old) were exposed to nominal concentrations of 0.0427, 0.0939, 0.207, 0.455 and 1.0 mg test item/L; equivalent to 0.0222, 0.0488, 0.108, 0.237 and 0.520 mg a.s./L (accounting for 51.99 % purity of test item). The overall geometric mean measured concentrations of total metam-sodium (metam-sodium and its degradation product methyl isothiocyanate (MITC)) were 0.0184, 0.0430, 0.107, 0.293 and 0.546 mg/L (between 83 and 124 % of nominal values). The biological results are reported based on nominal and mean measured concentrations of total metam-sodium.
The study was considered valid as all validity criteria were met. The 48 hour EC_{50} value based on nominal concentrations was calculated to be 0.291 mg test item/L (95 % confidence limits: 0.207 - 0.336 mg test item/L). The 48 hour EC_{50} value based on mean measured total metam-sodium concentrations was calculated to be 0.166 mg/L (95 % confidence limits: 0.107 - 0.199 mg/L). The no observed effect concentration (NOEC) value was determined to be 0.207 mg test item/L (nominal); 0.107 mg/L (mean measured total metam-sodium).

Materials and methods:

Test substance:	Metam-sodium (technical grade), batch no: E4227, chemical purity: 51.99 % w/v metam-sodium
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	First instar, < 24 hours old
Type of test:	Semi-test toxicity test (medium renewal after 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0.0427, 0.0939, 0.207, 0.455 and 1.0 mg metam-sodium (technical grade)/L; equivalent to 0 (control), 0.0222, 0.0488, 0.108, 0.237 and 0.520 mg a.s./L
Dilution medium:	Elendt M4 medium
Number of organisms per group:	5 daphnids per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 20.0 – 20.5 °C (test vessels), 20.3 – 20.8 °C (additional vessel containing dilution medium, which was monitored continuously) dissolved oxygen: 92 - 99 % O_2 saturation pH: 7.86 – 7.98 hardness: 221 mg/L $CaCO_3$ alkalinity: 93 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	Stock cultures of *Daphnia magna* were maintained in glass vessels containing approximately 0.5 to 0.8 litres of Elendt M4 culture medium in a temperature-controlled laboratory at nominally 20 ± 2

	°C. A photoperiod of 16 hours light: 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The culture medium was renewed three times each week. Cultures were fed daily with a suspension of the unicellular green algae, *Pseudokirchneriella subcapitata*, to provide nominally 0.1 to 0.2 mg carbon per daphnid, per day, except during the initial three days when a slightly lower ration was given. Culture conditions ensure that the stock animals reproduce by parthenogenesis. The day before the start of the study, all juvenile *Daphnia magna* were removed from the laboratory cultures. The following morning, juveniles produced by the gravid (egg-bearing) adult *Daphnia magna* were removed from the culture vessels and held in a separate holding vessel; these animals, which were less than 24 hours old, were used in the test. The test organisms were maintained, and the tests conducted, in Elendt M4 medium. The medium was prepared in deionised, reverse osmosis water. The medium used to prepare the aqueous stock solutions was de-oxygenated by bubbling helium through it for 20 minutes before use. On each day of preparation, the test substance (12.5 mg) was dissolved in de-oxygenated Elendt M4 medium (100 mL) in a volumetric flask to provide a concentrated, aqueous stock solution at a nominal concentration of 125 mg/L. The contents of the flask were shaken before being used directly at the highest test concentration or serially diluted with deoxygenated Elendt M4 medium to provide the intermediate stock media at the four lower concentrations. The aqueous stock test media were prepared in subdued light and the vessels were kept in black plastic bags to exclude light. An aliquot (1 mL) of the appropriate stock solution was added to dilution medium (124 mL) in Wheaton vials, to provide test media at nominal concentrations of 0.0427, 0.0939, 0.207, 0.455 and 1.0 mg test item/L; equivalent to 0.0222, 0.0488, 0.108, 0.237 and 0.520 mg a.s./L (accounting for 51.99 % purity of test item). The additional vessels for analytical samples were prepared in the same way as the test media. Two control groups were tested in addition to the five test item concentrations: a control group containing dilution medium (Elendt M4 medium) and an additional control group prepared by adding an aliquot (1 mL) of de-oxygenated dilution medium to control dilution medium. The time between weighing out the test substance and the start of the definitive test (when the last daphnid neonate was added to the appropriate test vessel) was approximately 95 minutes. Twenty daphnids, four replicates of five animals per vessel, were exposed in each control and test group. In each replicate five daphnids were assigned at random to completely filled test vessels to give a loading of approximately 25 mL medium per organism. The test vessels were sealed leaving no headspace. Daphnids were exposed to the test or control conditions for a period of 48 hours with renewal of test media at 24 hours.
Test item analysis:	Metam-sodium is known to be unstable in water and forms a volatile, unstable breakdown product (methyl isothiocyanate;

MITC). The test media were prepared from a series of aqueous stock solutions that had been made in Elendt M4 medium that had been de-oxygenated in an attempt to reduce the rate at which metam-sodium degrades. The exposure concentrations were monitored by measuring the concentrations of the active substance metam-sodium using an HPLC-UV method of analysis and the concentrations of the breakdown product MITC using a GC-NPD method of analysis. Two samples (125 mL) were taken from the freshly-prepared control and test media at 0 and 24 hours. For aged media at 24 and 48 hours, the contents of the specific vessels established from each group were taken for analysis. These vessels were filled at 0 and 24 hours as for the test vessels but no daphnids were added. On each occasion, one of the samples was analysed and the other was stored in a refrigerator in case further analysis was required.

Test results were expressed in terms of total metam-sodium calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium.

Observations: The numbers of mobile and immobile daphnids were counted approximately 24 and 48 hours after the start of the study. Daphnids were considered to be immobile if they were unable to swim within approximately 15 seconds following gentle agitation of the test vessel.

Statistical evaluation: Statistical analysis was performed using the SAFEStat LD_{50} application (version 1.3), SAS 9.1.3 (SAS Institute, 2002). Test results were expressed in terms of both nominal test item concentration and mean measured total metam-sodium concentration (calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium). The no observed effect concentration (NOEC) value was derived by direct inspection of the data on the immobility of daphnids. An incidence rate of more than 10 % was considered to be significant.

Findings:

Analytical results: In samples of freshly prepared media, the measured concentrations of total metam-sodium ranged between 47 and 163 % of their nominal values, with metam-sodium present as the primary constituent at nominal concentrations of 0.207 to 1.0 mg test item/L. At nominal concentrations of 0.0427 and 0.0939 mg test item/L, MITC was the primary constituent, with metam-sodium concentrations ranging from less than the limit of detection of the analytical method (0.005 mg/L) to 0.0199 mg/L. In samples of aged (24 hours old) media, the measured concentrations of total metam-sodium ranged between 67 and 124 % of their nominal, with MITC as the main constituent; no metam-sodium was detected. These results were not unexpected as both metam-sodium and MITC were known to be unstable in water at the concentrations employed in the test. The overall geometric mean measured concentrations of total metam-

	sodium (technical grade) were 0.0184, 0.0430, 0.107, 0.293 and 0.546 mg/L (between 83 and 124 % of nominal values). The biological results are reported based on nominal and mean measured concentrations of total metam-sodium (calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium).
Immobilisation:	After 48 hours, the lowest measured concentration at which 100 % immobility occurred was 0.455 mg/L (as nominal metam-sodium (technical grade); equivalent to 0.293 mg/L as measured total metam-sodium), and the highest concentration at which no immobilisation was noted was 0.0939 mg/L (as nominal metam-sodium (technical grade); equivalent to 0.0430 mg/L as measured total metam-sodium). The 48 hour EC_{50} value for the immobilisation of *Daphnia magna* based on nominal concentrations of metam-sodium (technical grade) was calculated to be 0.291 mg test item/L (95 % confidence limits: 0.207 - 0.336 mg test item/L). The 48 hour EC_{50} value based on mean measured total metam-sodium concentrations was calculated to be 0.166 mg/L (95 % confidence limits: 0.107 - 0.199 mg/L). The no observed effect concentration (NOEC) value was determined to be 0.207 mg test item/L (nominal metam-sodium (technical grade)); 0.107 mg/L (mean measured total metam-sodium).

Table B.2.9.2.2-19: Measured concentrations of total metam-sodium in test media following 48 hours of exposure of *Daphnia magna* to metam-sodium (technical grade)

Nominal concentrations		Measured concentrations (mg total metam-sodium/L)[b] Values in parentheses represent % of nominal concentration				
mg test item/L	mg metam-sodium/L[a]	0 h (fresh)	24 h (aged)	24 h (fresh)	48 h (aged)	Overall geometric mean
Control		n.d.	n.d.	n.d.	n.d.	n.d.
deoxygenated control		n.d.	n.d.	n.d.	n.d.	n.d.
0.0427	0.0222	0.0172 (77 %)	0.0233 (105 %)	0.0104 (47 %)	0.0276 (124 %)	0.0184 (83 %)
0.0939	0.0488	0.0253 (52 %)	0.0587 (120 %)	0.0379 (78 %)	0.0606 (124 %)	0.0430 (88 %)
0.207	0.108	0.0885 (82 %)	0.0719 (67 %)	0.171 (158 %)	0.120 (111 %)	0.107 (99 %)
0.455	0.237	0.306 (129 %)	0.246 (104 %)	0.376 (159 %)	0.262 (111 %)	0.293 (124 %)
1.0	0.520	0.423 (81 %)	0.472 (91 %)	0.850 (163 %)	0.525 (101 %)	0.546 (105 %)

[a] *Based on 51.99 % w/v purity of test item*
[b] *Sum of the concentrations of metam-sodium and MITC expressed as metam-sodium*
n.d. – None detected (< LOD of 0.005 mg/L for metam-sodium)

Table B.2.9.2.2-20: Cumulative immobilisation of *Daphnia magna*, exposed to metam-sodium (technical grade) for 48 hours under semi-static test conditions

Concentration (mg test item/L)	% of immobilised *Daphnia magna*

Nominal metam-sodium (technical grade)	Mean measured total metam-sodium	24 hours	48 hours
Control	n.d.	0	0
deoxygenated control	n.d.	0	0
0.0427	0.0184	0	0
0.0939	0.0430	0	0
0.207	0.107	0	5
0.455	0.293	25	100
1.0	0.546	100	100

n.d. – None detected (< LOD of 0.005 mg/L for metam-sodium)

Assessment and conclusions:

The acute toxicity of metam-sodium (technical grade) to *Daphnia magna* was assessed under sealed, semi-static (24 hour renewal period) exposure conditions over a period of 48 hours in accordance with OECD test guideline 202 (2004).
The overall geometric mean measured concentrations of total metam-sodium (metam-sodium and its degradation product methyl isothiocyanate (MITC)) were 0.0184, 0.0430, 0.107, 0.293 and 0.546 mg/L (between 83 and 124 % of nominal values). The biological results are reported based on nominal and mean measured concentrations of total metam-sodium.
The study was considered valid as all validity criteria were met. The 48 hour EC_{50} value for the immobilisation of *Daphnia magna* based on nominal concentrations of metam-sodium (technical grade) was calculated to be 0.291 mg test item/L (95 % confidence limits: 0.207 - 0.336 mg test item/L). The 48 hour EC_{50} value based on mean measured total metam-sodium concentrations was calculated to be 0.166 mg/L (95 % confidence limits: 0.107 - 0.199 mg/L). The no observed effect concentration (NOEC) value was determined to be 0.207 mg test item/L (nominal metam-sodium (technical grade)); 0.107 mg/L (mean measured total metam-sodium).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
EC_{50} (*Daphnia magna*, 48 h) = 0.166 mg metam-sodium/L (mean measured)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment. Method validation is acceptable according to the guidance SANCO/3029/99 rev. 4 on analytical validation.
Assessment and conclusion by Lainco:
The study is acceptable.
EC_{50} (*Daphnia magna*, 48 h, semi-static) = 0.166 mg metam-sodium/L (mean measured total metam-sodium and MITC) (95 % confidence limits: 0.107 – 0.199 mg/L)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 92 – 99 % O_2 saturation, equivalent to 8.36 – 9.00 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.

Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/81 (Taminco) and KCA 4.1.2/14 (Lainco), for further details).
EC_{50} (*Daphnia magna*, 48 h, semi-static) = 0.166 mg metam-sodium/L (based on mean measured concentrations total metam-sodium)
NOEC (*Daphnia magna*, 48 h, semi-static) = 0.107 mg metam-sodium/L (based on mean measured concentrations total metam-sodium)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.4.2/01
Report author:	[redacted]
Report year:	1992
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to the mysid, *Mysidopsis bahia*, under flow-through test conditions.
Report No.:	J9201013c
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-3
Deviations from current test guideline:	Deviations from OPPTS 850.1035 (1996) Photoperiod: 16:8 hours light:dark (recommendation: 14:10 hours light:dark) Test temperature occasionally exceeded the protocol range of 22 ± 1 °C. Temperature changes were gradual in nature and control mysids did not appear stressed. Therefore, this deviation did not affect the outcome of the test.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Metam Sodium Task Force)

Study Summary:

In a flow-through acute toxicity test, saltwater mysids (*Americamysis bahia*) were exposed for 96 hours to nominal concentrations of 0 (negative control), 1.3, 2.2, 3.6, 6.0 and 10.0 mg Potassium N-methyldithiocarbamate (metam-potassium)/L (equivalent to the mean measured concentrations of 0, 1.23, 1.78, 3.34, 5.92 and 8.93 mg metam-potassium/L) in collected seawater.
After 96 hours of exposure, no immobilisation was observed in the control. Until 24 hours after initial exposure no immobilisation was observed at any test concentration. After 48 hours of exposure, at the concentration level of 8.93 mg metam-potassium/L (mean measured) the number of immobilised organisms was 19. After 72 hours of exposure, at the concentration levels of 3.34, 5.92 and 8.93 mg metam-potassium/L the number of immobilized organisms was 5, 5 and 20, respectively. After 96 hours of exposure, at the concentration levels of 1.78, 3.34, 5.92 and 8.93 mg metam-potassium/L the number of immobilised organisms was 9, 12, 11 and 20, respectively.

The 96 hour LC_{50} value was 2.19 mg metam-potassium/L (mean measured concentration), with a 95 % confidence interval of 1.23 to 8.9 mg metam-potassium/L. The NOEC was 1.23 mg metam-potassium/L (mean measured concentration).

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Saltwater mysid (*Americamysis bahia*)
Age of organisms:	Post-larval mysids, < 24 hours old
Feeding:	Mysids were fed live brine shrimp nauplii daily during the test to reduce cannibalism
Type of test:	Flow-through toxicity test (7.0 volume replacements per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 1.3, 2.2, 3.6, 6.0 and 10.0 mg a.s./L
Number of organisms per group:	10 mysids per replicate, 2 replicates for the control and per treatment group
Time of exposure:	96 hours In-life dates: April 17th to 21st 1992
Test conditions:	temperature: 19.6 – 23.2 °C dissolved oxygen: 7.2 – 7.6 mg/L O_2, 81 – 85 % O_2 saturation pH: 8.2 – 8.4 salinity: 20 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: 367 – 667 lux
Test procedure:	Post-larval (< 24 hours old) mysids *Americamysis bahia* were exposed by groups of 10 in 2 replicates in a flow-through acute test for 96 hours to nominal concentrations of 0 (negative control), 1.3, 2.2, 3.6, 6.0 and 10.0 mg metam-potassium/L (equivalent to the mean measured concentrations of 0, 1.23, 1.78, 3.34, 5.92 and 8.93 mg metam-potassium/L) in collected seawater. Test concentrations were determined in a range-finding test.
Test item analysis:	Quantitation of metam-potassium was performed by high performance liquid chromatography (HPLC) using a UV-VIS detector and the external standard technique. The method was validated by fortifying aliquots of filtered saltwater with metam-potassium at the upper and lower ends of the expected concentration range to be used in the toxicity tests.
Observations:	Survival of mysids was monitored daily and any dead mysids were removed. Any abnormalities in the behavior or physical appearance of the mysids were also noted. Test water quality was monitored each day during the test.
Statistical evaluation:	The LC_{50} values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for LC_{50} values determined by non-linear interpolation were calculated by binomial probability.

Findings:

Analytical results: The diluter functioned properly and test concentrations remained stable during the entire definitive test. The mean measured concentrations during the 96 hour exposure ranged from 1.23 to 8.9 mg metam-potassium/L and from 81 to 99 % of nominal.

Mortality: Mortality of mysids exposed for 96 hours to metam-potassium ranged from 0 % at a mean measured test concentration of 1.23 mg metam-potassium/L to 100 % at a test concentration of 8.93 mg metam-potassium/L. Mortality in the dilution water controls was 0 %. The 96 hour LC_{50} based on measured metam-potassium concentrations was 2.19 mg metam-potassium/L with 95 % confidence limits of 1.23 to 8.93 mg metam-potassium/L. The no-observed-effect concentration (NOEC) was 1.23 mg metam-potassium/L (measured concentration).

Table B.2.9.2.2-21: Mortality of the saltwater mysid (*Americamysis bahia*), exposed to metam-potassium for 96 hours under flow-through test conditions

Nominal concentration of test item mg metam-potassium/L)	Mean measured concentration of test item (mg metam-potassium/L)	No. of *Americamysis bahia*	Cumulative number dead (percent mortality)			
			24 hours	48 hours	72 hours	96 hours
control	0	20	0 (0)	0 (0)	0 (0)	0 (0)
1.3	1.23	20	0 (0)	0 (0)	0 (0)	0 (0)
2.2	1.78	20	0 (0)	0 (0)	0 (0)	9 (45)
3.6	3.34	20	0 (0)	0 (0)	5 (25)	12 (60)
6.0	5.92	20	0 (0)	0 (0)	5 (25)	11 (55)
10.0	8.93	20	0 (0)	19 (95)	20 (100)	20 (100)

Assessment and conclusions:

Saltwater mysids (*Americamysis bahia*) were exposed for 96 hours under flow-through conditions to five mean measured concentrations of metam-potassium ranging from 1.3 to 10.0 mg metam-potassium/L (equivalent to the mean measured concentrations of 1.23 to 8.93 mg metam-potassium/L). The 96 hour LC_{50} value was 2.19 mg metam-potassium/L (mean measured concentration), with a 95 % confidence interval of 1.23 to 8.93 mg metam-potassium/L. The NOEC was 1.23 mg metam-potassium/L (mean measured concentration).

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable as supplementary data.

Endpoints:

EC_{50} (*Americamysis bahia*, 96 h) = 2.19 mg metam-potassium/L (mean measured)

NOEC (*Americamysis bahia*, 96 h) = 1.23 mg metam-potassium/L (mean measured)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to aquatic invertebrates in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 7.2 - 7.6 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/46, for further details).
EC_{50} (*Americamysis bahia*, 96 h, flow-through) = 2.19 mg metam-potassium/L (based on mean measured concentrations)
NOEC (*Americamysis bahia*, 96 h, flow-through) = 1.23 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.4.2/02
Report author:	[redacted]
Report year:	1993
Report title:	Potassium N-methyldithiocarbamate (PNMDC): Acute Effect On New Shell Growth of the Eastern Oyster, *Crassostrea virginica*.
Report No.:	J9201013e
Document No.:	-
Guidelines followed in study:	Subdivision E, US EPA Guideline No. 72-3
Deviations from current test guideline:	Deviations from OPPTS 850.1025 (1996) Valve height of test organisms: 23 – 33 mm (recommendation: 30 – 50 mm) Temperature: 24.9 – 26.7 °C (recommendation: 20 ± 5 °C) Temperature was not monitored continuously throughout the exposure as recommended due to an instrument malfunction. This protocol deviation was not considered significant and in the scientific judgement of the study director, did not affect the test results.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some limitations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: Metam Sodium Task Force)

Study Summary:

In a flow-through acute toxicity test, Eastern oysters (*Crassostrea virginica*) were exposed for 96 hours to nominal concentrations of 0 (negative control), 3.9, 6.5, 11, 18, 30 and 50 mg Potassium N-methyldithiocarbamate (metam-potassium)/L (equivalent to the mean measured concentrations of < 0.64 (negative control), 3.22, 5.62, 9.54, 17.1, 28.1 and 49.0 mg metam-potassium/L) in collected seawater.
Survival of oysters was 100 % in the control and all metam-potassium test concentrations. After 96 hours of exposure to metam-potassium, mean new shell growth ranged from 1.71 mm at 3.22 mg metam-potassium/L to 0.00 mm at 49.0 mg metam-potassium/L; mean new shell growth in the dilution water control was 2.32 mm. Percentage decrease in new shell growth of metam-potassium-exposed oysters as compared to the dilution water control ranged from 26 % at 3.22 mg metam-potassium/L to 100 % at 49.0 mg metam-potassium/L. Mean new shell growth was statistically reduced from that measured for the control oysters in all metam-potassium concentrations.
Based on Eastern Oyster (*Crassostrea virginica*) shell growth data, the 96 hour EC_{50} value was 6.45 mg metam-potassium/L (mean measured concentration), with a 95 % confidence interval of 3.22 to 9.53 mg metam-potassium/L. The NOEC was not determined because it was less than the lowest concentration tested (3.22 mg metam-potassium/L).

Materials and methods:

Test substance:	Potassium N-methyldithiocarbamate (metam-potassium), batch no: 1A-1275, chemical purity: 54.0 % (analysed)
Test species:	Eastern Oyster (*Crassostrea virginica*)
Age of organisms:	juveniles Oysters used for testing were from a population with an umbo to distal valve length of approximately 23 to 33 mm (mean length of 27.3 mm and standard deviation of 2.8 mm). Upon receipt, approximately 2 to 5 mm of shell growth was removed from the edge of each oyster with a high speed grinder to create a clean flat surface from which to measure new shell growth. These oysters were then added to a control tank in the test system for 2 days prior to test initiation.
Feeding:	Oysters were fed the marine diatom *Skeletonema costatum* twice per day during the test in an effort to boost new shell growth
Type of test:	Flow-through toxicity test (89 volume replacements per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 3.9, 6.5, 11, 18, 30 and 50 mg a.s./L
Number of organisms per group:	20 oysters for the control and per treatment group
Time of exposure:	96 hours In-life dates: October 16th to 20th 1992
Test conditions:	temperature: 24.9 – 26.7 °C dissolved oxygen: > 6.0 mg/L O_2, > 88 % O_2 saturation pH: 8.0 – 8.2 salinity: 30 - 34 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: 800 – 975 lux

Test procedure: Eastern oyster (*Crassostrea virginica*) juveniles (approximately 23 – 33 mm valve height umbo – distal edge) were exposed by groups of 20 in a flow-through acute test for 96 hours to nominal concentrations of 0 (negative control), 3.9, 6.5, 11, 18, 30 and 50 mg metam-potassium/L (equivalent to the mean measured concentrations of < 0.64 (negative control), 3.22, 5.62, 9.54, 17.1, 28.1 and 49.0 mg metam-potassium/L) in collected seawater. Test concentrations were determined in a range-finding test.
Prior to initiating the study, any new shell growth that was present on the oysters was removed and the 96 hour test was initiated with the impartial addition of oysters, by twos, to all test containers until 20 oysters were distributed to each test container.

Test item analysis: Quantitation of metam-potassium Technical was performed by liquid chromatography (LC) using a UV/VIS detector and the external standard technique. The method was validated by fortifying unfiltered saltwater with metam-potassium Technical at levels encompassing the upper and lower ends of the expected range of concentrations to be utilized in toxicity tests.

Observations: Observations of test solutions and survival of oysters was monitored daily. Test water quality was monitored each day during the test. Salinity and temperature of the dilution water was measured once daily in the dilution water control. Dissolved oxygen concentrations and pH were measured daily in all test treatments.

Statistical evaluation: The EC_{50} values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit, and non-linear interpolation. Confidence limits for EC_{50} values determined by non-linear interpolation were calculated by binomial probability.

Findings:

Analytical results: One day prior to test initiation, measured concentrations ranged from 81 to 89 % of nominal. At test initiation, measured concentrations ranged from 2.76 to 42.1 mg metam-potassium/L and 58 to 84 % of nominal; the stock concentrations were 77 to 111 % of nominals. Mean measured concentrations of metam-potassium ranged from 3.22 to 49.0 mg metam-potassium/L and from 83 to 98 % of nominal concentrations.

Mortality: Survival of oysters was 100 % in the control and all metam-potassium test concentrations. After 96 hours of exposure to metam-potassium, mean new shell growth ranged from 1.71 mm at 3.22 mg metam-potassium/L to 0.00 mm at 49.0 mg metam-potassium/L; mean new shell growth in the dilution water control was 2.32 mm. Percentage decrease in new shell growth of metam-potassium-exposed oysters as compared to the dilution water control ranged from 26 % at 3.22 mg metam-potassium/L to 100 % at 49.0 mg metam-potassium/L. Mean new shell growth was statistically reduced from that measured for the control oysters in all metam-potassium concentrations. For more details please refer to the following table.

Table B.2.9.2.2-22: New shell growth of the Eastern Oyster (*Crassostrea virginica*), exposed to metam-potassium for 96 hours under flow-through test conditions

Nominal concentration of test item mg metam-potassium/L)	Mean measured concentration of test item (mg metam-potassium/L)	No. of *Crassostrea virginica*	New shell growth (mm)		
			Treatment mean (SD)	Difference from control	Percent Change[a]
control	< 0.64	20	2.32 (0.62)	-	-
3.9	3.22	20	1.71 (0.32)	-0.61	-26
6.5	5.62	20	1.28 (0.54)	-1.04	-45
11	9.54	20	0.83 (0.67)	-1.49	-64
18	17.1	20	0.55 (0.63)	-1.77	-76
30	28.1	20	0.05 (0.22)	-2.27	-98
50	49.0	20	0.00 (0.00)	-2.32	-100

a Reduction in new shell growth, as compared to the pooled controls, is statistically significant at α = 0.05

Assessment and conclusions:

Based on Eastern Oyster (*Crassostrea virginica*) shell growth data, the 96 hour EC_{50} value was 6.45 mg metam-potassium/L (mean measured concentration), with a 95 % confidence interval of 3.22 to 9.53 mg metam-potassium/L. The NOEC was not determined because it was less than the lowest concentration tested (3.22 mg metam-potassium/L).

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable as supplementary data.

Endpoints:

EC_{50} (*Crassostrea virginica*, 96 h) = 6.45 mg metam-potassium/L (mean measured)

NOEC (*Crassostrea virginica*, 96 h) < 3.22 mg metam-potassium/L (mean measured)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment, and therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco. The previously evaluated studies with metam-potassium were not considered as critical endpoints for acute toxicity to aquatic invertebrates in the previous evaluation (and hence were not listed in the previous EFSA conclusion).

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The following validity criterium of OECD Guideline 202 were met:

- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: > 6.0 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.

Therefore, this study is still considered acceptable.

The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/50, for further details).

EC_{50} (*Crassostrea virginica*, 96 h, flow-through) = 6.45 mg metam-potassium/L (based on mean measured concentrations)

NOEC (*Crassostrea virginica*, 96 h, flow-through) < 3.22 mg metam-potassium/L (based on mean measured concentrations)
Since the test item is metam-potassium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Studies with MITC

Data point:	KCA 8.2.4.1/04
Report author:	[redacted]
Report year:	2002
Report title:	Effect of Methyl-iso-thiocyanate (MITC) in the Immobility of *Daphnia magna* STRAUS in a 48 Hour Semi-Static, Acute Toxicity Test.
Report No.:	58330
Document No.:	2002/1006188
Guidelines followed in study:	OECD Guideline 202 (1984) EEC Directive 79/831, Annex V, Part C2 (1990) Subdivision E, US EPA Guideline No. 72-2 and OPPTS 850.1010 (1996)
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): Temperature: 21.0 – 23.5 °C (recommendation: 18 – 22 °C ± 1 °C)
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Method validation not fully in line with the SANCO/3029/99 rev.4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation. Therefore, the study is considered acceptable and relied upon in risk assessment.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

In this study the acute toxicity of Methyl-iso-thiocyanate (MITC) to the waterflea *Daphnia magna* has been determined. The investigation was carried out following OECD guideline 202. Daphnids were exposed by groups of 5 in 4 replicates for 48 hours to a dilution water control, a solvent control and to nominal concentrations of 0.05, 0.089, 0.158, 0.281 and 0.5 mg MITC/L corresponding to 0.026, 0.05, 0.115, 0.132 and 0.304 mg MITC/L (mean measured). Acetone was used as a solvent with a maximum concentration of 0.1 mL/L.
Mean measured MITC concentrations were in a range of 47 % to 73 % of nominal. Therefore, biological results were based on mean measured concentrations. No mortality or signs of intoxication were observed in the control groups and at MITC concentrations ≤ 0.05 mg/L. All daphnids died in treatment groups ≥ 0.115 mg MITC/L. The determined EC_{50} (48 h) was 0.076 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 408208/1, chemical purity: 99.6 %

Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	Neonates, < 24 hours old
Type of test:	Semi-static toxicity test (medium renewal after 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control: acetone), 0.05, 0.089, 0.158, 0.281 and 0.50 mg MITC/L
Number of organisms per group:	5 daphnids per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: July 2001
Test conditions:	temperature: 21.0 – 23.5 °C dissolved oxygen: 8.3 – 8.9 mg/L O_2, 94 – 101 % O_2 saturation pH: 8.04 – 8.24 total hardness: 2.43 – 2.47 mmol/L alkalinity: 0.87 mmol/L photoperiod: 16 hours light and 8 hours dark light intensity: < 15000 lux
Test procedure:	Daphnids were exposed by groups of 5 in 4 replicates for 48 hours to a dilution water control, a solvent control and to nominal concentrations of 0.05, 0.089, 0.158, 0.281 and 0.5 mg Methyl-iso-thiocyanate (MITC)/L. MITC test concentrations were selected based on the results of a range finding test. Acetone was used as a solvent with a maximum concentration of 0.1 mL/L. A stock solution was prepared by dissolving 35.65 mg test substance in 3.56 mL acetone. This acetone solution was added to M4-Medium (50 µL to 500 ml M4-Medium). The stock solution was constantly stirred while appropriate amounts of it were taken for dilution to the desired test concentrations. After 24 hours, a water renewal was carried out. A stock solution was prepared by adding 44 mg of the test substance to 4.44 mL acetone and further dilution with test medium (50 µL of acetone test solution in 500 mL M4-water).
Test item analysis:	At the beginning of the test, 24 hours after test initiation, immediately after renewal of the test medium, and at the end of the test samples from each concentration were taken for analytical verification of nominal concentrations. MITC was quantified by reversed phase HPLC using a Spherisorb ODS II HPLC column with acetonitrile/water as mobile phase. Quantification is achieved by UV detection of MITC at 248 nm and external calibration using MITC as reference substance. The limit of quantification in this study was determined to be 5 µg/L. Prior to the analytical determination, the aqueous MITC samples were acidified with 0.1% (v/v) hydrochlorid acid in order to minimize the possible further hydrolysis of MITC. An aliquot of 2 mL of the acidified test samples was injected directly onto the HPLC column. Each sample was injected twice. Standard solutions in the concentration range of approximately 0.02 mg/L up to approximately 2.3 mg MITC/L were prepared and used as instrumentation test and quantification. The resulting calibration curve with a correlation coefficient of > 0.9999 showed the linear dependence of the detector signal from the concentrations

of the standard solutions. The accuracy of the calibration function was checked by a separate second weight of the reference substance. The identity of the test substance was confirmed by comparison of the mean HPLC retention time of the reference item substance with the mean retention time of the corresponding test substance peak in the test samples.

Observations: Daphnids were observed after 24 and 48 hours to determine the number of immobile organisms and other signs of toxicity in each treatment group.
The pH and oxygen in one replicate at each concentration were determined at 0 hour, water renewal after 24 hours and 48 hours after test initiation. Hardness and specific conductance in the dilution water were measured at the beginning of the test and at water renewal.

Statistical evaluation: The mathematical determination of the EC_{50} (48 h) was calculated as geometric mean of EC_0 and EC_{100} = $\sqrt{(EC_0 \times EC_{100})}$; the 24 h EC_{50} was determined by Spearman-Karber analysis using a PC and the commercial software "TOXSTAT 3.5" (WEST, Inc., USA).

Findings:

Analytical results: At test initiation, the recoveries for MITC were in a range between 67 and 97.1 %. 24 hours after test initiation, the recoveries for the test substance went down to values between 23 and 35.4 %, which can be attributed to the known hydrolytic instability of MITC. Immediately after the renewal of the test medium, recoveries of 78.4 and 85.9 % were found for MITC, which again went down to values between 31.1 and 41.2 % after additional 24 hours. Detailed results of the measured MITC concentrations are listed in the table below. The biological results were based on mean measured concentrations.

Immobilisation: The concentrations response curve was very steep. No mortality or signs of intoxication were observed in the control groups and at MITC concentrations ≤ 0.05 mg/L. All daphnids died in treatment groups ≥ 0.115 mg MITC/L. Detailed results of immobilisation are presented in the table below.
The determined EC_{50} (48 h) was 0.076 mg MITC/L.
Additional effect values were:
EC_0 (48 h): 0.050 mg/L
EC_{100} (48 h): 0.115 mg/L
EC_{50} (24 h): 0.165 mg/L

Table B.2.9.2.2-23: Mean measured concentrations of MITC of two samples taken at test initiation (0 hours (new)), after 24 hours (old), after medium renewal (24 hours (new)) and at test termination (48 hours (old)) in M4 water samples of the *Daphnia* acute test

Nominal concentration of test item (mg MITC/L)	Measured concentrations (mg MITC/L)				Mean	% of nominal
	0 h (new)	24 h (old)	24 h (new)	48 h (old)		
Control	n.d.	n.d.	n.d.	n.d.	-	-
solvent control	n.d.	n.d.	n.d.	n.d.	-	-

0.05	0.0335	0.0115	0.0427	0.0156	0.026	52
0.089	0.0636	0.0315	0.0712	0.0327	0.05	56
0.158	0.2129	0.0703	0.1239	0.0511	0.115	73
0.281	0.1533	0.0534	0.2356	0.0873	0.132	47
0.5	0.4326	0.1474	0.4294	0.2058	0.304	61

n.d.: not detected

Table B.2.9.2.2-24: Immobility of *Daphnia magna*, exposed to MITC for 48 hours under semi-static test conditions

Nominal concentration of test item (mg MITC/L)	Mean measured concentration of test item (mg MITC/L)	Immobile								Total after 24 hours		Total after 48 hours	
		After 24 hours				After 48 hours							
		1	2	3	4	1	2	3	4	Nb.	%	Nb.	%
Control	control	0	0	0	0	0	0	0	0	0	0	0	0
solvent control	solvent control	0	0	0	0	0	0	0	0	0	0	0	0
0.05	0.026	0	0	0	0	0	0	0	0	0	0	0	0
0.089	0.05	0	0	0	0	0	0	0	0	0	0	0	0
0.158	0.115	0	0	0	0	5	5	5	5	0	0	20	100
0.281	0.132	2	1	2	3	5	5	5	5	8	40	20	100
0.5	0.304	5	5	5	5	5	5	5	5	20	100	20	100

Assessment and conclusions:

Results of this investigation indicate that MITC has a nominal 48 hour EC_{50} of 0.076 mg/L (mean measured) in a semi-static acute toxicity test with *Daphnia magna*.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
EC_{50} (*Daphnia magna*, 48 h) = 0.076 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the SSD derivation.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
EC_{50} (*Daphnia magna*, 48 h, semi-static) = 0.076 mg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.3 – 8.9 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is still considered acceptable.

No further information on the analytical method used.
EC_{50} (*Daphnia magna*, 48 h, semi-static) = 0.076 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.1/06
Report author:	[redacted]
Report year:	2019a
Report title:	Methyl isothiocyanate (MITC) – Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test.
Report No.:	IES Study 20180074
Document No.:	-
Guidelines followed in study:	OECD No. 202 (2004) Method C.2 of Commission Regulation (EC) No. 440/2008
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities (exception: pre-test for verification of the stability of the test item in ethanol (non-GLP))
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: Kanesho Soil Treatment, letter of co-ownership by Taminco is included, study may be used by Taminco without restriction in Europe only) (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

The acute toxicity of the test item Methyl isothiocyanate (MITC) *to Daphnia magna* was determined in a 48 hour test.
The test was run under flow-through conditions for a test period of 48 hours. At the start of the test, 20 daphnids each were exposed to test media containing the test item at different concentrations. The daphnids were observed for visible abnormalities and immobility.
For the dosage of the test item in test water, the solvent ethanol was used. The nominal concentrations of the test item of 5.0, 14, 39, 110 and 307 µg/L were tested. Additionally, a control and a solvent control were tested in parallel.
In the test medium samples from the analysed test media of nominal 5.0 to 307 µg/L taken at the start of the test, at day 1 and at day 2, the measured concentrations of Methyl isothiocyanate (MITC) were in the range of 80 – 102 % of the nominal values. This shows the constant exposure of the daphnids during the test period of 48 hours.
Up to the test item concentration of 35 µg/L (mean measured), no toxic effects were observed in the daphnids until test end. At the next higher test item concentration of 101 µg/L, visible abnormalities were observed after two days and six daphnids were immobilised. At the highest test item concentration of 290 µg/L visible abnormalities were observed after one day and ten daphnids were immobilised, while all daphnids were immobilised after two days.
The 48 hour EC_{50} was 134 µg MITC/L (based on nominal concentrations). The 48 hour NOEC was 39 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	First instar, 6 - 24 hours old
Type of test:	Flow-through toxicity test The frequency of test medium preparation cycles was 12 minutes, i.e. 5 cycles per hour. The high frequency of test medium preparation reduced the risk of losses of test item by evaporation in the mixing vessel. As 400 mL test vessels were used, this test medium flow resulted in a 30-fold theoretical test medium exchange rate per day.
Applied concentrations:	Nominal test concentrations: 0 (dilution water control), 0 (solvent control 60 µL/L ethanol), 5.0, 14, 39, 110 and 307 µg MITC/L
Dilution medium:	Reconstituted test water (ISO Test water) according to OECD 202
Number of organisms per group:	5 daphnids per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 20 °C dissolved oxygen: 8.8 – 9.3 mg/L O_2 pH: 7.6 hardness: 250 mg/L $CaCO_3$ alkalinity: 0.8 mmol/L photoperiod: 16 hours light and 8 hours dark light intensity: 10 – 12 µmol m^{-2} s^{-1}
Test procedure:	For determination of the appropriate test item concentrations for the main test, a range-finding test was performed. The test was performed under the same experimental conditions (flow-through) compared to the main test. For each treatment, two replicates with 5 daphnids each were used. Based on the results of the range finding test, the following nominal concentrations of the test item were tested in the main test: 5.0, 14, 39, 110 and 307 µg/L. Additionally, a control and a solvent control (60 µL/L ethanol) were tested in parallel (test water without test item). The test was run under flow-through conditions. For continuous dosing of the test item into test water a computer-controlled automated flow-through dosing system was used. The whole dosing system consisted of individual dosing units for each treatment. For the dosing of the test item into test water, concentrated application solutions of the test item were prepared for each test item concentration. For each treatment, 20 daphnids were randomly distributed into 4 replicates of 5 daphnids each.
Test item analysis:	For the measurement of the actual concentrations of the test item, duplicate samples were taken from all test media and from the solvent control at the start of the test and after 24 and 48 hours.

	Immediately after sampling, to each test medium sample about 20 g of sodium chloride were added. Then, the samples were extracted with internal standard solution. The organic phase was separated and stored frozen until analysis was performed. The analytical method used is gas chromatography with mass spectrometric detection (GC/MS).
Observations:	The immobility of the daphnids was determined by visual inspection after 24 and 48 hours of exposure. Those daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. Observations were also performed for visible abnormalities (e.g. discolouration, surface trapping, incoherent swimming pattern, reduced swimming, etc.)
Statistical evaluation:	The NOEC, LC_0 and LC_{100} were determined directly from the Raw Data. The 24 and 48 hour LC_{50} at the observation times and the 95 % confidence limits were calculated as far as possible by the Trimmed Spearman-Karber procedure. Statistical analysis was performed using ToxRat Professional®.

Findings:

Analytical results:	No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the test period of 48 hours. In the test medium samples from the analysed test media of nominal 5.0 to 307 µg/L taken at the start of the test, at day 1 and at day 2, the measured concentrations of Methyl isothiocyanate (MITC) were in the range of 80 – 102 % of the nominal values. This shows the constant exposure of the daphnids during the test period of 48 hours. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test concentration. The biological results are based on the mean measured test item concentrations. Additionally, as all measured test item concentrations were between 80 and 120 % of nominal, the biological results are presented based on nominal test item concentrations.
Immobilisation:	During the first 24 hours of the test, no immobilised test organisms were determined in the controls and the test item concentrations up to and including mean measured 101 µg/L (nominal 110 µg/L). At 290 µg/L (nominal 307 µg/L), the immobilisation rate was 50 % and visible abnormalities were observed at the daphnids which were assessed to be mobile. After 48 hours exposure, no immobilised test organisms were determined in the solvent control and up to and including the test item concentration of mean measured 35 µg/L (nominal 39 µg/L). In the control, one daphnia was immobile but this immobility rate of 5 % was within the 10 % immobility rate tolerated by the test guideline in the control. At mean measured 101 µg/L (nominal 110 µg/L) the immobility rate was 30 % and visible abnormalities were observed at the daphnids which were

assessed to be mobile. At the highest test concentration of mean measured 290 µg/L (nominal 307 µg/L) all daphnids were immobile after 48 hours of exposure.
The biological endpoints (based on mean measured concentrations) are summarised as follows:
24 hour EC_{50} = 287 µg/L (95 % confidence limits: 227 – 362 µg/L)
24 hour EC_0 and NOEC = 101 µg/L
24 hour EC_{100} > 290 µg/L
48 hour EC_{50} = 124 µg/L (95 % confidence limits: 100 – 154 µg/L)
48 hour EC_0 and NOEC = 35 µg/L
48 hour EC_{100} = 290 µg/L
The biological endpoints (based on nominal test item concentrations) are summarized as follows:
24 hour EC_{50} = 307 µg/L (95 % confidence limits: 244 – 387 µg/L)
24 hour EC_0 and NOEC = 110 µg/L
24 hour EC_{100} > 307 µg/L
48 hour EC_{50} = 134 µg/L (95 % confidence limits: 109 – 167 µg/L)
48 hour EC_0 and NOEC = 39 µg/L
48 hour EC_{100} = 307 µg/L

Table B.2.9.2.2-25: Mobility and visible abnormalities for *Daphnia magna*, exposed to MITC for 48 hours under flow-through test conditions

Nominal test item concentration (µg/L)	Mean measured test item concentration (µg/L)	No. of daphnids per replicate	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
			No.	(%)	No.	(%)
Control	Control	5 5 5 5	0 0 0 0	0	0 0 1 0	5
Solvent control	Solvent control	5 5 5 5	0 0 0 0	0	0 0 0 0	0
5.0	4.9	5 5 5 5	0 0 0 0	0	0 0 0 0	0
14	13	5 5 5 5	0 0 0 0	0	0 0 0 0	0
39	35	5 5 5 5	0 0 0 0	0	0 0 0 0	0
110	101	5 5 5 5	0 0 0 0	0	1 (D, F) 2 (D, F) 2 (D, F) 1 (D, F)	30
307	290	5 5 5 5	3 (D, F) 2 (D, F) 2 (D, F) 3 (D, F)	50	5 5 5 5	100

In parenthesis: Adverse effects observed in the mobile daphnids
A: daphnids trapped at the water surface
B: daphnids sticking together
C: antennae sticking together
D: daphnids discolored/pale
E: spina stuck
F: reduced swimming ability

Assessment and conclusions:

The test item Methyl isothiocyanate (MITC) had acute toxic effects on *Daphnia magna* in a 48 hour flow-through test.
The 48 hour EC_{50} was calculated to be 134 µg/L with 95 % confidence limits of 109 – 167 µg/L (based on nominal concentrations).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):

The study is acceptable.
Endpoints:
EC_{50} (*Daphnia magna*, 48 h) = 0.134 mg MITC/L (nominal)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable and relied upon.
Assessment and conclusion by Lainco:
The study is acceptable.
EC_{50} (*Daphnia magna*, 48 h, flow-through) = 134 µg MITC/L (nominal) (95 % confidence limits: 109 – 167 µg MITC/L)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.8 – 9.3 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/69 (Taminco) and KCA 4.1.2/17 (Lainco), for further details).
EC_{50} (*Daphnia magna*, 48 h, flow-through) = 0.124 mg MITC/L (based on mean measured concentrations)
NOEC (*Daphnia magna*, 48 h, flow-through) = 35 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/03
Report author:	[redacted]
Report year:	2018a
Report title:	Methyl isothiocyanate (MITC) – Effect on First-Instar Larvae of *Chironomus riparius* in a 48-Hour Immobilization Test.
Report No.:	IES Study 20180117
Document No.:	-
Guidelines followed in study:	OECD No. 235 (2011)
Deviations from current test guideline:	Deviations from current OECD guideline 235 (2011): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities (exception: range-finding test and pre-test for verification of the stability of the test item)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

	(original Sponsor: Kanesho Soil Treatment, letter of co-ownership by Taminco is included, study may be used by Taminco without restriction in Europe only) (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

The acute toxicity of the test item Methyl isothiocyanate (MITC) to first-instar larvae of the midge *Chironomus riparius* was determined in a 48 hour semi-static test according to the OECD Guideline for Testing of Chemicals, No. 235 (2011).
The nominal test item concentrations tested were 6.25, 12.5, 25, 50 and 100 µg/L. Additionally, a control (test water without test item) was tested in parallel.
As the test item is a volatile substance, the test was performed using glass tubes completely filled (without headspace) with test medium that were tightly sealed with glass stoppers to avoid losses of test item by evaporation (closed system).
The measured concentrations of Methyl isothiocyanate (MITC) in the test media of the test concentrations from 6.25 to 100 µg/L at the start and the end of the two test medium renewal periods are shown in the table below. At the start of the two renewal periods, the test item concentrations were in the range of 80 to 123 % of the nominal values and between 78 and 123 % at the end of the two renewal periods. The mean measured test item concentrations over the test period of 48 hours were calculated as the arithmetic mean.
Up to the test item concentration of 23 µg/L (mean measured), no toxic effects were observed in the larvae until test end. At the next higher test item concentration of 46 µg/L, 25 % of the larvae were immobilised after 48 hours. At the highest test item concentration of 93 µg/L 70 % of the larvae were immobilised after 24 hours, while all larvae were immobilised after 48 hours.
The 48 hour EC_{50} was 55 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Midge (*Chironomus riparius*)
Age of organisms:	First-instar larvae, 2 days old
Type of test:	Semi-static toxicity test (daily medium renewal)
Applied concentrations:	Nominal test concentrations: 0 (control), 6.25, 12.5, 25, 50 and 100 µg MITC/L
Dilution medium:	Elendt M7 medium
Number of organisms per group:	5 midges per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 21 – 22 °C dissolved oxygen: 8.1 – 8.4 mg/L O_2 pH: 7.0 – 7.1 hardness: 250 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 770 – 830 lux
Test procedure:	The selection of the test concentrations was based on the results of an non-GLP range-finding test. The following nominal concentrations of Methyl isothiocyanate (MITC) were tested in the

	main test: 6.25, 12.5, 25, 50 and 100 µg/L (factor 2). A control (test water without test item) was tested in parallel. The test was conducted with daily medium renewal (semi-static). The test was performed with first-instar larvae of the midge *Chironomus riparius*. For each treatment, 20 larvae were used, divided into 4 replicates of 5 larvae each. The test duration was 48 hours.
Test item analysis:	For the determination of the actual test item concentrations, duplicate samples were taken from each treatment at the start and the end of each test medium renewal. No insoluble test materials were observed in the test vessels. All samples were taken at the mid-depth of the test vessels. Immediately after sampling, to each test medium sample about 20 g of sodium chloride were added. Then, the samples were extracted with intern standard solution. The organic phase was separated and stored frozen until analysis was performed. The analytical method used is gas chromatography with mass spectrometric detection (GC/MS).
Observations:	The immobility of the larvae or abnormalities at the test animals were visually determined after 24 and 48 hours of exposure. Those organisms which were not able to change their position (by crawling or swimming movements) within 15 seconds after mechanical stimulation, e.g. by subjecting the larvae to a gentle stream of water from a Pasteur pipette or agitation of the test vessel are considered to be immobilised. The pH values, water temperature and oxygen concentrations of the test media were measured and recorded in each test concentration and the control at the start and the end of each test medium-renewal period. In addition, the appearance of the test media was visually controlled and documented at the same time.
Statistical evaluation:	The 24 hour EC_{50} could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead, the 24 hour EC_{50} was calculated by linear interpolation of the immobility between the two consecutive test concentrations with 0 % and 70 % immobility on a semi-logarithmic scale (the 95 % confidence limits could not be determined). The 48 hour EC_{50} and the 95 % confidence limits were calculated by the Trimmed Spearman-Karber method for estimating median lethal concentrations, since the estimation based on the Probit or logit models was not valid (p(F) > 0.05). Statistical analysis were performed using ToxRat Professional®.

Findings:

Analytical results:	No remarkable observations were made concerning the appearance of the test media. All test media were clear throughout the entire test duration. The measured concentrations of Methyl isothiocyanate (MITC) in the test media of the test concentrations from 6.25 to 100 µg/L

	at the start and the end of the two test medium renewal periods are shown in the table below. At the start of the two renewal periods, the test item concentrations were in the range of 80 to 123 % of the nominal values and between 78 and 123 % at the end of the two renewal periods. The mean measured test item concentrations over the test period of 48 hours were calculated as the arithmetic means. The biological results are based on the mean measured concentrations.
Immobilisation:	During the first 24 hours of the test, no immobilised test organisms were detected in the control or at the mean measured test item concentrations of 5.5 to 46 µg/L. At the highest test concentration of 93 µg/L, the immobilisation rate was 70 %. The 24 hour EC_{50} of the test item was calculated to be 76 µg/L. The 24 hour NOEC was 46 µg/L. After the test period of 48 hours, no immobilised test organisms were determined in the control or any of the test media up to and including the concentration of 23 µg/L. At the test concentrations of 46 and 93 µg/L, 25 and 100 % of the larvae were found to be immobile, respectively. The 48 hour EC_{50} of the test item was calculated to be 55 µg/L with 95 % confidence limits from 48 to 63 µg/L. The 48 hour NOEC was 23 µg/L, since no immobilisation was observed up to and including this test concentration.

Table B.2.9.2.2-26: Nominal and mean measured concentrations of MITC of two samples taken at start and end of each medium renewal of the *Chironomus riparius* acute test

Nominal Test Item Concentration [µg/L]	Measured Test Item Concentrations at the Start of the Renewal Periods (Day 0 / Day 1) [µg/L]	Measured Test Item Concentrations at the End of the Renewal Periods (Day 1 / Day 2) [µg/L]	Mean Measured Concentration of the Test Item (Arithmetic Mean) [µg/L]
6.25	5.81 / 4.99	6.22 / 4.89	5.5
12.5	15.4 /10.5	15.4 / 10.8	13
25	25.8 / 22.3	23.7 / 20.7	23
50	48.9 / 46.5	46.7 / 43.4	46
100	96.2 / 91.6	96.4 / 88.1	93

Table B.2.9.2.2-27: Immobility of the midges (*Chironomus riparius*), exposed to MITC for 48 hours under semi-static test conditions

Nominal Test Item Concentration [µg/L]	Measured Test Item Concentration [µg/L]	No. of Larvae Tested	Immobilized Larvae after 24 hours		Immobilized Larvae after 48 hours	
			No.	[%]	No.	[%]
control	--	20	0	0	0	0
6.25	5.5	20	0	0	0	0
12.5	13	20	0	0	0	0
25	23	20	0	0	0	0
50	46	20	0	0	5	25
100	93	20	14	70	20	100

Assessment and conclusions:

The test item Methyl isothiocyanate (MITC) had acute toxic effects on *Chironomus riparius* in a 48 hour semi-static test.
The 48 hour EC_{50} was calculated to be 55 µg/L; the 95 %-confidence limits were 48 and 63 µg/L (based on mean measured concentrations).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
EC_{50} (*Chironomus riparius*, 48 h) = 55 µg MITC/L (mean measured)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The study is acceptable.
EC_{50} (*Chironomus riparius*, 48 h, semi-static) = 55 µg MITC/L (mean measured) (95 % confidence limits: 48 – 63 µg MITC/L)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 235 were met:

- the immobility in the controls should not exceed 15 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.1 – 8.4 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 235 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/71 (Taminco) and KCA 4.1.2/20 (Lainco), for further details).
EC_{50} (*Chironomus riparius*, 48 h, semi-static) = 55 µg MITC/L (based on mean measured concentrations)
NOEC (*Chironomus riparius*, 48 h, semi-static) = 23 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/04
Report author:	[redacted]
Report year:	2014c
Report title:	Methyl isothiocyanate (MITC) – A 48-hour static acute toxicity test with the midge (*Chironomus riparius*).
Report No.:	657A-106
Document No.:	-
Guidelines followed in study:	OECD No. 235 (2011)
Deviations from current test guideline:	Deviations from current OECD guideline 235 (2011): None
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a static acute toxicity test, first instar larvae midges (*Chironomus riparius*) were exposed in groups of 5 organisms in 4 replicates per treatment 48 hours to nominal concentrations of 0 (negative control), 0.20, 0.30, 0.44, 0.67, 1.0 and 1.5 mg Methyl Isothiocyanate (MITC)/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.084, 0.12, 0.17, 0.28, 0.37 and 0.66 mg MITC/L) in collected freshwater.
Midges in the negative control and the 0.084, 0.12 and 0.17 mg MITC/L (mean measured) treatment groups all appeared normal throughout the test, with no immobility or other signs of toxicity observed. Percent immobility at test termination in the 0.28, 0.37 and 0.66 mg MITC/L (mean measured) treatment groups was 0 %, 60 % and 100 %, respectively. Signs of toxicity observed in the 0.28, 0.37 and 0.66 mg MITC/L (mean measured) treatment groups included lethargy.
The 48 hour EC_{50} value for *Chironomus riparius*, based on the mean measured test concentrations of MITC and immobility, was estimated to be 0.36 mg MITC/L, with 95 % confidence limits of 0.28 to 0.66 mg MITC/L. The no-immobility concentration was 0.28 mg MITC/L and the NOEC was 0.17 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 97.2 % (according to certificate of analysis)
Test species:	Midge (*Chironomus riparius*)
Age of organisms:	First-instar larvae, 1 - 4 days old after hatching
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.20, 0.30, 0.44, 0.67, 1.0 and 1.5 mg MITC/L
Number of organisms per group:	5 midges per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: April 15th to 17th 2014
Test conditions:	temperature: 20.1 – 20.7 °C dissolved oxygen: 8.9 – 9.1 mg/L O_2 pH: 8.1 – 8.5 hardness: 144 mg/L $CaCO_3$

	alkalinity: 178 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 512 lux
Test procedure:	First instar larvae midges (*Chironomus riparius*) (of approximately 1 - 4 days after hatching) were exposed by groups of 5 organisms in 4 replicates per treatment in a static acute test for 48 hours to nominal concentrations of 0 (negative control), 0.20, 0.30, 0.44, 0.67, 1.0 and 1.5 mg MITC/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.084, 0.12, 0.17, 0.28, 0.37 and 0.66 mg MITC/L) in collected freshwater. Test concentrations were determined in a range-finding test.
Test item analysis:	Duplicate water samples were collected from the batches of test solutions prepared for each treatment and control group at the beginning of the test and from two replicate test chambers in each control and treatment group at 48 hours (± 1 hour) to measure concentrations of the test substance. The samples were diluted in freshwater, as necessary and transferred to VOA vials with no head space. Samples were submitted for analysis by gas chromatography with flame ionization detection (GC/FID).
Observations:	All organisms were observed periodically to determine the number of immobile organisms in each treatment group. Immobile organisms were not able to change their position (by crawling or swimming movements) within 15 seconds after mechanical stimulation (e.g., by subjecting the larvae to a gentle stream of water from a pipette or agitation of the test vessel). The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 2, 24 and 48 hours after test initiation. Temperature was measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Dissolved oxygen and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Hardness and alkalinity in the dilution water at the beginning of the test were measured.
Statistical evaluation:	Nonlinear interpolation was used to calculate the 48 hour EC_{50} value and binominal probability was used to calculate the 95 % confidence interval. Due to the method used to calculate the 48 hour EC_{50} value, the slope of the dose response curve could not be calculated. Since there was < 50 % immobility at 24 hours, the 24 hour EC_{50} value, as well as the no-immobility concentration and NOEC, were determined by visual interpretation of the immobility and observation data.

Findings:

Analytical results:	Measured concentrations of the samples collected at test initiation ranged from approximately 84 to 92 % of nominal, indicating the solutions were prepared at the proper concentrations. Measured concentrations of the samples collected at test termination ranged from approximately 9 to 31 % of nominal. When measured concentrations of the samples

were averaged, the mean measured test concentrations for this study were 0.084, 0.12, 0.17, 0.28, 0.37 and 0.66 mg MITC/L, representing 42, 40, 39, 42, 37 and 44 % of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Immobilisation: Midges in the negative control and in the 0.084, 0.12 and 0.17 mg MITC/L (mean measured) treatment groups all appeared normal throughout the test, with no immobility or other signs of toxicity observed. Percent immobility at test termination in the 0.28, 0.37 and 0.66 mg MITC/L (mean measured) treatment groups was 0 %, 60 % and 100 %, respectively. Signs of toxicity observed in the 0.28, 0.37 and 0.66 mg MITC/L (mean measured) treatment groups included lethargy.

Table B.2.9.2.2-28: Immobility of the midges (*Chironomus riparius*), exposed to MITC for 48 hours under static test conditions

Nominal concentration of test item (mg MITC/L)	Mean measured concentration of test item (mg MITC/L)	No. of *Chiro-nomus riparius*	Immobilization at						Cumulative immobilization [%]
			2 hours		24 hours		48 hours		
			No.	Obs.*	No.	Obs.*	No.	Obs.*	
control	< LOQ	20	0	20AN	0	20AN	0	20AN	0
0.20	0.084	20	0	20AN	0	20AN	0	20AN	0
0.30	0.12	20	0	20AN	0	20AN	0	20AN	0
0.44	0.17	20	0	20AN	0	20AN	0	20AN	0
0.67	0.28	20	0	20AN	0	20AN	0	3C 17AN	0
1.0	0.37	20	0	20AN	0	20AN	12	8C	60
1.5	0.66	20	0	20AN	0	3C 17AN	20	-	100

** AN = Appear Normal; C = Lethargy*

Assessment and conclusions:

The 48 hour EC_{50} value for *Chironomus riparius*, based on the mean measured test concentrations of MITC and immobility was estimated to be 0.36 mg MITC/L, with 95 % confidence limits of 0.28 to 0.66 mg MITC/L. The no-immobility concentration was 0.28 mg MITC/L and the NOEC was 0.17 mg MITC/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

EC_{50} (*Chironomus riparius*, 48 h) = 0.36 mg MITC/L (mean measured)

EC_0 (*Chironomus riparius*, 48 h) = 0.28 mg MITC/L (mean measured)

Analytical method:

Method validation not fully in line with the guidance SANCO/3029/99 rev.4 on analytical validation, but given that the endpoint is in line with the state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 235 were met:

- the immobility in the controls should not exceed 15 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.9 – 9.1 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 235 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/51, for further details), taking into consideration the lower level achieved in the study with *Hyalella azteca*.
EC_{50} (*Chironomus riparius*, 48 h, static) = 0.36 mg MITC/L (based on mean measured concentrations)
EC_0 (*Chironomus riparius*, 48 h, static) = 0.28 mg MITC/L (based on mean measured concentrations)
NOEC (*Chironomus riparius*, 48 h, static) = 0.17 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/05
Report author:	[redacted]
Report year:	2014a
Report title:	MITC: Acute Toxicity to the Larval Phase of the Midge *Chironomus riparius*.
Report No.:	PQB0023
Document No.:	-
Guidelines followed in study:	Procedure 235 (*Chironomus* sp., Acute Immobilisation Test) of the "Guidelines for Testing of Chemicals" of the Organisation for Economic Co-operation and Development (OECD: 2001)
Deviations from current test guideline:	Deviations from current OECD guideline 235 (2011): None
Previous evaluation:	No, not previously submitted at EU level Evaluated and accepted to support Lainco S.A.'s products at Step 2 under Directive 91/414/EEC (Final RR Part B6, 2017, zRMS Spain) IIIA 10.2.2.2/03
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Lainco S.A. (letter of co-ownership by Taminco is included, study may be used by Taminco without restriction for registration purposes)

Study Summary:

The acute toxicity of MITC to the larval phase of *Chironomus riparius* was assessed in a 48 hour laboratory test under semi-static conditions (renewal of test media after 24 hours) in accordance with OECD test guideline 235 (2011). Groups of twenty larval chironomids were exposed for 48 hours to MITC, at nominal concentrations of 1.82, 4.01, 8.82, 19.4, 42.7, 93.9, 207, 455 and 1000 µg MITC/L.

Overall geometric mean measured concentrations of 1.93, 4.58, 10.3, 22.5, 51.5, 104, 235, 431 and 1140 µg MITC/L. After 48 hours, cumulative immobilisation and unaccounted for larvae values were 5, 5, 40, 10, 35, 15, 20, 20, 55, 95 and 100 % in the control, solvent control, 1.93, 4.58, 10.3, 22.5, 51.5, 104, 235, 431 and 1140 µg MITC/L (mean measured) treatment groups, respectively. The 48 hour EC_{50} value was calculated to be 90.6 µg MITC/L (mean measured) (95 % confidence limits: 48.9 - 183 µg MITC/L). The no observed effect concentration (NOEC) value was determined to be 104 µg MITC/L (mean measured).

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Midge (*Chironomus riparius*)
Age of organisms:	First-instar larvae
Type of test:	Semi-static toxicity test (medium renewal after 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (dilution water control), 0 (solvent control 100 µL/L acetone), 1.82, 4.01, 8.82, 19.4, 42.7, 93.9, 207, 455 and 1000 µg MITC/L
Number of organisms per group:	5 midges per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 20.5 – 22.2 °C dissolved oxygen: 88 – 106 % air saturation saturation pH: 8.19 – 8.37 hardness: 150 – 200 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 630 lux
Test procedure:	First-instar chironomid larvae (*Chironomus riparius*), were exposed by groups of 5 organisms in 4 replicates in each control and test group, for a period of 48 hours, with renewal of the test media after 24 hours. Based on the results of a range-finding test, the definitive test employed nominal concentrations of 1.82, 4.01, 8.82, 19.4, 42.7, 93.9, 207, 455 and 1000 µg MITC/L.
Test item analysis:	The test concentrations of MITC were measured using GC-NPD liquid chromatography. At 0 and 24 hours during the definitive test, two samples (20 mL) were taken from the freshly-prepared control and test media. At 24 and 48 hours, the contents of the vessels from each group were pooled and further samples were taken for analysis. Samples were stabilized by the addition of sodium chloride and ethyl acetate. On each occasion, one of the samples was analysed and the other was stored in a freezer in case further analysis was required.
Observations:	The survival of the organisms was determined. Chironomid larvae were considered to be immobile if they did not react to gentle stimulation for 15 seconds. The numbers of mobile and immobile chironomid larvae were counted approximately 24 and 48 hours after the start of the study. Environmental conditions were monitored throughout the test. The temperature, pH and dissolved oxygen levels were recorded for each group in fresh media at 0 and 24 hours, and in pooled expired media at 24 and 48 hours.

Statistical evaluation: Statistical analysis was performed using the SAFEStat LD_{50} application (version 1.5), SAS 9.1.3. Test results were expressed in terms of the mean measured concentrations.
The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the organisms. An incidence rate of more than 15 % was considered to be significant where a treatment-related trend in the data set was identified. In the study, immobility up to 20 % was considered not to be significant, because no treatment-related trend in the levels of immobility were observed over a wide range of concentrations.

Findings:

Analytical results: In samples of freshly prepared media (0 and 24 hours) the measured concentrations of MITCC ranged between 98 and 146 % of their nominal values. In samples of expired media (at 24 and 48 hours), the measured concentrations had decreased to between 72 and 111 % of their nominal values (between 58 and 82 % of their starting values). Based on a geometric mean, the overall measured concentrations of MITC were 1.93, 4.58, 10.3, 22.5, 51.5, 104, 235, 431 and 1140 µg MITC/L, and these values have been used in the determination of study end-points.

Immobilisation: After 48 hours, 95 % immobility was observed at 431 µg MITC/L with 100 % immobility observed at 1140 µg MITC/L. At 1.93 to 104 µg MITC/L, immobility ranged between 15 and 20 %; although slightly above 15 % (the maximum acceptable level of immobility for the control group), there was no treatment-related trend in the data. Therefore, the highest concentration with no significant effect was considered to be 104 µg MITC/L.

Table B.2.9.2.2-29: Immobility of the midges (*Chironomus riparius*), exposed to MITC for 48 hours under semi-static test conditions

Nominal concentration (µg MITC/L)	Measured concentrations (µg MITC/L)	Cumulative no. immobile *Chironomus riparius* after		Cumulative % immobile *Chironomus riparius* after	
		24 hours	48 hours	24 hours	48 hours
control	not detected	1	1	5	5
solvent control	not detected	1	1	5	5
1.82	1.93	7	8	35	40
4.01	4.58	2	2	10	10
8.82	10.3	7	7	35	35
19.4	22.5	3	3	15	15
42.7	51.5	3	4	15	20
93.9	104	3	4	15	20
207	235	8	11	40	55
455	431	5	19	25	95
1000	1140	20	20	100	100

Assessment and conclusions:

The 24 hour measured EC_{50} value for *Chironomus riparius* in a semi-static study was calculated to be 565 µg MITC/L (mean measured) (95 % confidence limits of 190 and 6990 µg MITC/L). The 24 hour NOEC value was determined to be 104 µg MITC/L (mean measured).
The 48 hour measured EC_{50} value for *Chironomus riparius* in a semi-static study was calculated to be 90.6 µg MITC/L (mean measured) (95 % confidence limits of 48.9 and 183 µg/L). The 48 hour NOEC value was determined to be 104 µg MITC/L (mean measured).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
EC_{50} (*Chironomus riparius*, 48 h) = 0.0906 mg MITC/L (mean measured) (95 % confidence limits: 0.0489 – 0.183 mg MITC/L)
Analytical method:
The method is acceptable for the quantification of MITC in aquatic arthropod medium.
Assessment and conclusion by Lainco:
The study is acceptable.
EC_{50} (*Chironomus riparius*, 48 h, semi-static) = 0.0906 mg MITC/L (mean measured) (95 % confidence limits: 0.0489 – 0.183 mg MITC/L)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 235 were met:
- the immobility in the controls should not exceed 15 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L (34 % ASV) in control and test vessels (measured: 88 – 106 % air saturation value)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 235 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/82 (Taminco) and KCA 4.1.2/19 (Lainco), for further details).
EC_{50} (*Chironomus riparius*, 48 h, semi-static) = 90.6 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/06
Report author:	[redacted]
Report year:	2014a
Report title:	Methyl isothiocyanate (MITC): A 48-hour static acute toxicity test with the freshwater amphipod (*Hyalella azteca*).
Report No.:	657A-108
Document No.:	-
Guidelines followed in study:	The test protocol based on procedures outlined in the OECD Guideline 202 and U.S. EPA OPPTS Number 850.1010
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004):

	Test organism: *Hyalella azteca* (recommendation: *Daphnia magna*) Diet: Tetramin® (recommendation : no feeding)
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a static acute toxicity test, 6 days old freshwater amphipod (*Hyalella azteca*) were exposed by groups of 5 organisms in 4 replicates per treatment for 48 hours to nominal concentrations of 0 (negative control), 1.0, 3.0, 9.0, 28, 85 and 286 µg Methyl Isothiocyanate (MITC)/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.59, 1.2, 3.5, 11, 36 and 104 µg MITC/L) in collected freshwater.
No clinical signs of toxicity were observed for amphipods in the negative control and the 0.59 and 1.2 µg MITC/L treatment groups. Percent mortality in the negative control and in the 0.59 and 1.2 µg MITC/L treatment groups was 10 %, 15 % and 10 %, respectively. While mortality in the 0.59 and 1.2 µg MITC/L treatment groups was not dose responsive and was comparable to mortality in the control, a treatment-related effect could not be precluded. Percent mortality at test termination in the 3.5, 11, 36 and 104 µg MITC/L treatment groups was 50 %, 70 %, 100 % and 100%, respectively. Lethargy was observed in amphipods in the 104 µg MITC/L treatment group. All other amphipods in the treatment groups appeared normal throughout the test.
The 48 hour LC_{50} value for *Hyalella azteca*, based on the mean measured test concentrations of Methyl Isothiocyanate (MITC) and mortality, was estimated to be 3.8 µg MITC/L, with 95 % confidence limits of 2.6 to 5.8 µg MITC/L. The slope of the concentration-response curve was 1.8. The no-mortality concentration and the NOEC were both < 0.59 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 97.2 % (according to certificate of analysis)
Test species:	Freshwater amphipod (*Hyalella azteca*)
Age of organisms:	Approximately 6 days old
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 1.0, 3.0, 9.0, 28, 85 and 256 µg MITC/L
Number of organisms per group:	5 amphipods per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: April 9th to 11th 2014
Test conditions:	temperature: 22.7 – 23.5 °C dissolved oxygen: 8.2 – 8.6 mg/L O_2 pH: 8.1 – 8.5 hardness: 148 mg/L $CaCO_3$ alkalinity: 182 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 966 lux

Test procedure:	Six days old freshwater amphipods (*Hyalella azteca*) were exposed by groups of 5 organisms in 4 replicates per treatment in a static acute test for 48 hours to nominal concentrations of 0 (negative control), 1.0, 3.0, 9.0, 28, 85 and 286 µg MITC/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.59, 1.2, 3.5, 11, 36 and 104 µg MITC/L) in collected freshwater. Test concentrations were determined in a range-finding test.
Test item analysis:	Duplicate samples were collected from the batches of test solutions prepared for each treatment and control group at the beginning of the test and from each test chamber at 48 hours (± 1 hour) to measure concentrations of the test substance. The samples were diluted in freshwater, as necessary and transferred to VOA vials with no head space. Samples were submitted for analysis by gas chromatography with flame ionization detection (GC/FID).
Observations:	All organisms were observed periodically for mortality and clinical signs of toxicity. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus. The numbers of individuals exhibiting abnormal behavior also were evaluated. Observations were made approximately 5, 24 and 48 hours after test initiation. Temperature was measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Temperature also was monitored continuously during the test in a container of water placed adjacent to the test chambers. Dissolved oxygen and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Hardness and alkalinity in the dilution water at the beginning of the test were measured.
Statistical evaluation:	Probit analysis was used to calculate the 48 hour LC_{50} value and the 95 % confidence interval. Nonlinear interpolation was used to calculate the 24 hour LC_{50} value and binominal probability was used to calculate the 24 hour 95 % confidence interval. The no-mortality concentration and NOEC were determined by visual interpretation of the mortality and observation data.

Findings:

Analytical results:	Measured concentrations of the samples collected at test initiation ranged from approximately 88 to 94 % of nominal, indicating the solutions were prepared at the proper concentrations. Measured concentrations of the samples collected at test termination ranged from approximately < LOQ to 22 % of nominal. When measured concentrations of the samples were averaged, the mean measured test concentrations for this study were 0.59, 1.2, 3.5, 11, 36 and 104 µg MITC/L, representing 59, 40, 39, 39, 42 and 41 % of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.
Immobilisation:	No clinical signs of toxicity were observed for amphipods in the negative control and the 0.59 and 1.2 µg MITC/L treatment groups. Percent mortality in the negative control and the 0.59

and 1.2 µg MITC/L treatment groups was 10 %, 15 % and 10 %, respectively. While mortality in the 0.59 and 1.2 µg MITC/L treatment groups was not dose responsive and was comparable to mortality in the control, a treatment-related effect could not be precluded. Percent mortality at test termination in the 3.5, 11, 36 and 104 µg MITC/L treatment groups was 50 %, 70 %, 100 % and 100 %, respectively. Lethargy was observed in amphipods in the 104 µg MITC/L treatment group. All other amphipods in the treatment groups appeared normal throughout the test.

Table B.2.9.2.2-30: Mortality of the freshwater amphipod (Hyalella azteca), exposed to MITC for 48 hours under static test conditions

Nominal concentration of test item (µg MITC/L)	Mean measured concentration of test item (µg MITC/L)	No. of *Hyalella azteca*	Mortality at						Cumulative mortality [%]
			5 hours		24 hours		48 hours		
			No.	Obs.*	No.	Obs.*	No.	Obs.*	
Control	< LOQ	20	0	20AN	0	20AN	2	18AN	10
1.0	0.59	20	0	20AN	0	20AN	3	17AN	15
3.0	1.2	20	0	20AN	0	20AN	2	18AN	10
9.0	3.5	20	0	20AN	0	20AN	10	10AN	50
28	11	20	0	20AN	1	19AN	12	6AN	70
85	36	20	0	20AN	0	20AN	20	-	100
256	104	20	0	20C	20	-	20	-	100

** AN = Appear Normal; C = Lethargy*

Assessment and conclusions:

The 48 hour LC_{50} value for *Hyalella azteca*, based on the mean measured test concentrations of MITC and mortality, was estimated to be 3.8 µg MITC/L, with 95 % confidence limits of 2.6 to 5.8 µg MITC/L. The slope of the concentration-response curve was 1.8. The no-mortality concentration and the NOEC were both < 0.59 µg MITC/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

LC_{50} (*Hyalella azteca*, 48 h) = 0.0038 mg MITC/L (mean measured)

LC_0 (*Hyalella azteca*, 48 h) < 0.00059 mg MITC/L (mean measured)

Analytical method:

Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 10 %)

- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.2 - 8.6 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/52, for further details).
There is uncertainty on the lower measured test concentrations, but since used in a SSD calculation, the endpoint is considered acceptable, though slight deficiencies.
LC_{50} (*Hyalella azteca*, 48 h, static) = 3.8 µg MITC/L (based on mean measured concentrations)
NOEC (*Hyalella azteca*, 48 h, static) < 0.59 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/07
Report author:	[redacted]
Report year:	2014b
Report title:	Methyl isothiocyanate (MITC): A 48-hour static acute toxicity test with the marine amphipod (*Leptocheirus plumulosus*).
Report No.:	657A-109
Document No.:	-
Guidelines followed in study:	The test protocol based on procedures outlined in the OECD Guideline 202 and U.S. EPA OPPTS Number 850.1010
Deviations from current test guideline:	Deviations from current OECD guideline 202 (2004): Test organism: *Leptocheirus plumulosus* (recommendation: *Daphnia magna*)
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a static acute toxicity test, marine amphipod (*Leptocheirus plumulosus*) were exposed by groups of 5 organisms in 4 replicates per treatment for 48 hours to nominal concentrations of 0 (negative control), 0.013, 0.033, 0.083, 0.21, 0.52 and 1.3 mg Methyl Isothiocyanate (MITC)/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.0096, 0.020, 0.052, 0.12, 0.25 and 0.63 mg MITC/L) in collected seawater.
Amphipods in the negative control and the 0.0096, 0.020 and 0.052 mg MITC/L (mean measured) treatment groups all appeared normal throughout the test, with no mortality or other signs of toxicity observed, with the exception of one incidental mortality in the negative control at the 48 hour observations. Percent mortality at test termination in the 0.12, 0.25 and 0.63 mg MITC/L (mean measured) treatment groups was 25 %, 90 % and 100 %, respectively. Signs of toxicity observed in the 0.25 mg MITC/L (mean measured) treatment group included lethargy.
Based on mortality data, the 48 hour LC_{50} value for *Leptocheirus plumulosus* was 0.16 mg MITC/L, with a 95 % confidence interval of 0.13 to 0.19 mg MITC/L. The slope of the concentration-response curve was 6.3. The no-mortality concentration and the NOEC were both 0.052 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 97.0 % (according to certificate of analysis)
Test species:	Marine amphipod (*Leptocheirus plumulosus*)
Age of organisms:	Immature amphipods of approximately 2 to 4 mm in length
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.013, 0.033, 0.083, 0.21, 0.52 and 1.3 mg MITC/L
Number of organisms per group:	5 amphipods per replicate, 4 replicates for the control and per treatment group
Time of exposure:	48 hours In-life dates: May 7th to 9th 2014
Test conditions:	temperature: 22.1 – 22.6 °C dissolved oxygen: 7.2 – 7.9 mg/L O_2 (≥ 94 % air saturation value) pH: 7.9 – 8.1 salinity: 20 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: 531 lux
Test procedure:	Immature marine amphipods (*Leptocheirus plumulosus*) (of approximately 2 to 4 mm in length) were exposed by groups of 5 organisms in 4 replicates per treatment in a static acute test for 48 hours to nominal concentrations of 0 (negative control), 0.013, 0.033, 0.083, 0.21, 0.52 and 1.3 mg MITC/L (equivalent to the mean measured concentrations of < LOQ (negative control), 0.0096, 0.020, 0.052, 0.12, 0.25 and 0.63 mg MITC/L) in collected seawater. Test concentrations were determined in a range-finding test.
Test item analysis:	Duplicate water samples were collected from the batches of test solutions prepared for each treatment and control group at the beginning of the test and from two replicate test chambers in each control and treatment group at 48 hours (± 1 hour) to measure concentrations of the test substance. The analytical method consisted of diluting the samples with either freshwater or 10:90 (v/v) saltwater; freshwater and analyzed by gas chromatography with flame ionization detection (GC/FID).
Observations:	All organisms were observed periodically to determine the number of mortalities in each treatment group. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus. The numbers of individuals exhibiting signs of toxicity or abnormal behavior were also evaluated. Observations were made approximately 3, 24 and 48 hours after test initiation. Temperature was measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Temperature also was monitored continuously in an adjacent container of water. Dissolved oxygen and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hours during the test. Salinity was measured in each test chamber at the beginning and end of the test.

Statistical evaluation: Nonlinear interpolation was used to calculate the 24 hour LC_{50} value and binomial probability was used to calculate the 95 % confidence interval. Probit analysis was used to calculate the 48 hour LC_{50} value and the 95 % confidence interval. The no-mortality concentration and NOEC were determined by visual interpretation of the mortality and observation data.

Findings:

Analytical results: Measured concentrations of the samples collected at test initiation ranged from approximately 92.9 to 102 % of nominal, indicating the solutions were prepared at the proper concentrations. Mean measured concentrations of the samples collected at test termination ranged from < LOQ to 46.5 % of nominal. Mean measured concentration for each treatment group of the study was determined by taking the average of test substance concentration at test initiation and mean of replicate values at test termination. The results of the study were based on the mean measured concentrations.

Immobilisation: Amphipods in the negative control and the 0.0096, 0.02 and 0.052 mg MITC/L (mean measured) treatment groups all appeared normal throughout the test, with no mortality or other signs of toxicity observed, with the exception of one incidental mortality in the negative control at the 48 hour observations. Percent mortality at test termination in the 0.12, 0.25 and 0.63 mg MITC/L (mean measured) treatment groups was 25 %, 90 % and 100 %, respectively. Signs of toxicity observed in the 0.25 mg MITC/L (mean measured) treatment group included lethargy.

Table B.2.9.2.2-31: Mortality of the marine amphipod (*Leptocheirus plumulosus*), exposed to MITC for 48 hours under static test conditions

Nominal concentration of test item (mg MITC/L)	Mean measured concentration of test item (mg MITC/L)	No. of *Lepto-cheirus plumulosus*	Mortality at						Cumulative mortality [%]
			3 hours		24 hours		48 hours		
			No.	Obs.*	No.	Obs.*	No.	Obs.*	
control	< LOQ	20	0	20AN	0	20AN	1	19AN	5
0.013	0.0096	20	0	20AN	0	20AN	0	20AN	0
0.033	0.020	20	0	20AN	0	20AN	0	20AN	0
0.083	0.052	20	0	20AN	0	20AN	0	20AN	0
0.21	0.12	20	0	20AN	0	20AN	5	15AN	25
0.52	0.25	20	0	20AN	11	9C	18	2C	90
1.3	0.63	20	0	20AN	20	-	20	-	100

** AN = Appear Normal; C = Lethargy*

Assessment and conclusions:

The marine amphipod, *Leptocheirus plumulosus*, was exposed for 48 hours under static conditions to six mean measured concentrations of MITC ranging from 0.0096 to 0.63 mg MITC/L. The 48 hour LC_{50} value was 0.16 mg MITC/L, with a 95 % confidence interval of 0.13 to 0.19 mg MITC/L. The slope of

the concentration-response curve was 6.3. The no-mortality concentration and the NOEC were both 0.052 mg MITC/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Leptocheirus plumulosus*, 48 h) = 0.16 mg MITC/L (mean measured)
EC_0 (*Leptocheirus plumulosus*, 48 h) = 0.052 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 5 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 7.2 – 7.9 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, however it is still considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/56, for further details).
LC_{50} (*Leptocheirus plumulosus*, 48 h, static) = 0.16 mg MITC/L (based on mean measured concentrations)
NOEC (*Leptocheirus plumulosus*, 48 h, static) = 0.052 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/08
Report author:	[redacted]
Report year:	2011
Report title:	Methyl isothiocyanate (MITC): A 96-hour flow-through acute toxicity test with the saltwater midge (*Americamysis bahia*).
Report No.:	703A-101A
Document No.:	-
Guidelines followed in study:	U.S. EPA OPPTS 850.1035 (1996)
Deviations from current test guideline:	Deviations from OPPTS 850.1035 (1996): Minor deviations Photoperiod: 16 hours light: 8 hours darkness (recommendation: 14 hours light: 10 hours darkness)
Previous evaluation:	No, not previously submitted at EU level

	Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

In a flow-through acute toxicity test, saltwater mysids (*Americamysis bahia*) were exposed for 96 hours to nominal concentrations of 0 (negative and solvent control), 6.3, 13.0, 25.0, 50.0 and 100 µg Methyl Isothiocyanate (MITC)/L (equivalent to the mean measured concentrations of 0, 6.5, 12.0, 23.0, 48.0 and 87.0 µg MITC/L) in collected seawater.
After 96 hours of exposure, no immobilisation was observed in the controls. Until 24 hours after initial exposure no immobilisation was observed at any test concentration. After 48 hours of exposure, at the concentration levels of 25, 50 and 100 µg MITC/L the number of immobilised organisms was 2, 4 and 1, respectively. After 72 hours of exposure, at the concentration levels of 6.3, 13, 25, 50 and 100 µg MITC/L the number of immobilised organisms was 1, 1, 2, 6 and 1, respectively. After 96 hours of exposure, at the concentration levels of 6.3, 13, 25, 50 and 100 µg MITC/L the number of immobilised organisms was 2, 2, 5, 8 and 14, respectively. At this point in time the number of immobilised organisms in the solvent control was 1. Regarding the observations of abnormal behavior no occurrences were noted up to 48 hours after exposure. At 72 hours after exposure at the highest test concentration, 5 organisms were found to show lethargic signs. At 96 hours after exposure at the highest test concentration, 1 organism was found to show lethargic signs.
The 96 hour LC_{50} value for *Americamysis bahia* was 55 µg MITC/L, with a 95 % confidence interval of 42 to 79 µg MITC/L. The slope of the concentration-response curve was 2.749. The no-mortality concentration and the NOEC were both 12 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 99.7 % (according to certificate of analysis)
Test species:	Saltwater mysid (*Americamysis bahia*)
Age of organisms:	Juveniles, < 24 hours old
Type of test:	Flow-through toxicity test (10 volume replacements per day)
Applied concentrations:	Nominal test concentrations: 0 (dilution water control), 0 (solvent control 0.1 mL/L dimethylformamide), 6.3, 13, 25, 50 and 100 µg MITC/L
Number of organisms per group:	10 mysids per replicate, 2 replicates for the control, for the solvent control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 24.8 – 24.9 °C dissolved oxygen: 4.9 – 7.3 mg/L O_2 (≥ 67 % air saturation value) pH: 7.80 – 8.10 salinity: 20 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: 217 lux
Test procedure:	Juvenile *Americamysis bahia* were exposed by groups of 10 in 2 replicates in a flow-through acute test for 96 hours to nominal concentrations of 0 (negative and solvent control), 6.3, 13.0, 25.0, 50.0 and 100 µg MITC/L (equivalent to the mean measured

	concentrations of 0, 6.5, 12.0, 23.0, 48.0 and 87.0 µg MITC/L) in collected seawater. Test concentrations were determined in a range-finding test.
Test item analysis:	Samples were collected from one test chamber of each treatment and control group one day prior to the start of the test. Water samples were also collected from alternating replicate test chambers in each treatment and control group at the beginning of the test and at 48 and 96 hours (± 1 hour) to measure concentrations of the test substance. Two sets of samples were collected at 96 hours. One set was processed immediately for analysis, and the second set was stored refrigerated for possible further analysis. The samples were collected from mid-depth, acidified with two drops of phosphoric acid, placed in glass scintillation vials, and processed immediately for analysis. The analytical method consisted of diluting the samples in acidified saltwater and extracting with diethyl ether. An aliquot of each diethyl ether phase was transferred to autosampler vials and submitted for analysis by gas chromatography with mass selective detection (GC/MS).
Observations:	All organisms were observed periodically to determine the number of mortalities in each treatment group. The numbers of individuals exhibiting signs of toxicity or abnormal behavior were also evaluated. Observations were made approximately 6, 24, 48, 72 and 96 hours after test initiation. Temperature was measured in each test chamber at the beginning and end of the test. Temperature was also monitored continuously in one negative control test chamber. Dissolved oxygen and pH were measured in one replicate test chamber of each treatment and control group at the beginning of the test, at approximately 24 hour intervals during the test, and at the end of the test, with measurements typically alternating between replicates in each group at each measurement interval. Salinity was measured in the dilution water at test initiation and termination.
Statistical evaluation:	Probit analysis was used to calculate the 96 hour LC_{50} value and the 95 % confidence limits. Since there was < 50 % mortality at 24, 48, and 72 hour, the LC_{50} values, the no mortality concentrations, and the NOEC values for 24, 48, and 72 hours were determined by visual interpretation of the mortality and observation data.

Findings:

Analytical results:	Measured concentrations of the samples of the primary stock solution of MITC ranged from approximately 95 to 98 % of nominal, indicating that the test substance was being delivered to the test chambers at approximately the targeted nominal concentrations. Measured concentrations of the samples of the test solutions ranged from approximately 78 to 106 % of nominal. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 6.5, 12, 23, 48 and 87 µg MITC/L,

representing 103, 92, 92, 96 and 87 % of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Mortality and clinical signs of toxicity:

There was one mortality among mysids in the solvent control group observed at test termination. All other mysids in the negative control and solvent control groups appeared normal throughout the test. There was 10 % mortality in the 6.5 and 12 µg MITC/L treatment groups by test termination; all surviving mysids in these two treatment groups appeared normal throughout the test. Mortality in the 6.5 and 12 µg MITC/L was comparable to the solvent control group, and did not exceed the test guideline acceptance criteria of 10 % mortality for control organisms. Mortality of these two groups was also isolated to one of the two replicate test chambers in each group. Therefore, mortality in the 6.5 and 12 µg MITC/L treatment groups was not considered to be treatment-related and was excluded from calculation of the LC_{50} value. Percent mortality at test termination in the 23, 48 and 87 µg MITC/L treatment groups was 25 %, 40 % and 70 %, respectively. Lethargy was observed among mysids in the 87 µg MITC/L treatment group.

Since there was < 50 % mortality at 24, 48 and 72 hour, the LC_{50} values, the no-mortality concentrations, and the NOEC values for 24, 48 and 72 hours were determined by visual interpretation of the mortality and observation data.

The 96 hour LC_{50} was determined by Probit analysis to be 55 µg MITC/L, with a 95 % confidence interval of 42 to 79 µg MITC/L. The no-mortality concentration and the NOEC were 12 µg MITC/L. LC_{50} values at 24, 48, 72 and 96 hours were determined from the mortality data.

Daily observations of mortality and signs of toxicity observed during the test are presented in the table below.

Table B.2.9.2.2-32: Mortality of the saltwater midge (*Americamysis bahia*), exposed to MITC for 96 hours under flow-through test conditions

Nominal concentration of test item (µg MITC/L)	Mean measured concentration of test item (µg MITC/L)	No. of *Americamysis bahia*	Immobilisation at										Cumulative mortality [%]
			6 hours		24 hours		48 hours		72 hours		96 hours		
			No.	Obs. *	No.	Obs. *	No.	Obs. *	No.	Obs. *	No.	Obs. *	
control	< LOQ	20	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0
solvent control	< LOQ	20	0	20 AN	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5
6.3	6.5	20	0	20 AN	0	20 AN	0	20 AN	1	19 AN	2	18 AN	10
13	12	20	0	20 AN	0	20 AN	0	20 AN	1	19 AN	2	18 AN	10
25	23	20	0	20 AN	0	20 AN	2	18 AN	2	18 AN	5	15 AN	25
50	48	20	0	20 AN	0	20 AN	4	16 AN	6	14 AN	8	12 AN	40

100	87	20	0	20 AN	0	20 AN	1	19 AN	1	5 C, 14 AN	14	1 C, 5 AN	70

** AN = Appear Normal; C = Lethargy*

Assessment and conclusions:

Saltwater mysids (*Americamysis bahia*) were exposed for 96 hours under flow-through conditions to five mean measured concentrations of MITC ranging from 6.5 to 87 µg MITC/L. The 96 hour LC_{50} value was 55 µg MITC/L, with a 95 % confidence interval of 42 to 79 µg MITC/L. The slope of the concentration-response curve was 2.749. The no-mortality concentration and the NOEC were both 12 µg MITC/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Americamysis bahia*, 96 h) = 0.055 mg MITC/L (mean measured)
LC_0 (*Americamysis bahia*, 96 h) = 0.012 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 % in control and 5 % in solvent control)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 4.9 – 7.1 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/57, for further details).
LC_{50} (*Americamysis bahia*, 96 h, flow-through) = 55 µg MITC/L (based on mean measured concentrations)
NOEC (*Americamysis bahia*, 96 h, flow-through) = 12 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/09
Report author:	[redacted]
Report year:	2012b
Report title:	Methyl isothiocyanate (MITC): A 96-hour shell deposition test with Eastern Oyster (*Crassostrea virginica*).

Report No.:	703A-103B
Document No.:	-
Guidelines followed in study:	U.S. EPA OPPTS 850.1025
Deviations from current test guideline:	Deviations from OPPTS 850.1025: None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

In a flow-through acute toxicity test, Eastern Oysters (*Crassostrea virginica*) were exposed for 96 hours to nominal concentrations of 0 (negative and solvent control), 7.5, 15, 30, 60 and 120 µg Methyl Isothiocyanate (MITC)/L (equivalent to the mean measured concentrations of 6.4, 13, 24, 44 and 92 µg MITC/L) in collected seawater.
Survival of oysters was 100 % in the control and all MITC test concentrations. All oysters in the negative and solvent control groups, and in the 6.4, 13, 24 and 44 µg MITC/L treatment groups appeared normal throughout the 96 hour exposure period. Oysters in the 92 µg MITC/L treatment group were normal in appearance, however, there were observations of reduced feeding (oysters closed) for this group. After 96 hours, the mean shell deposition in the negative and solvent control groups was 2.1 and 2.4 mm, respectively. Mean shell deposition in the 6.4, 13, 24, 44 and 92 µg MITC/L treatment groups was 2.2, 1.8, 1.7, 0.8 and 0.04 mm, respectively. Dunnett's test indicated that there was a statistically significant decrease in shell deposition in the 44 and 92 µg MITC/L treatment groups in comparison to the pooled control.
Percent inhibition of Eastern Oyster (*Crassostrea virginica*) shell growth was calculated relative to the pooled control data. Inhibition of shell growth in the 6.4, 13, 24, 44 and 92 µg MITC/L treatment groups was 2.8 %, 20 %, 27 %, 64 % and 99 %, respectively. The 96 hour EC_{50} was calculated to be 42 µg MITC/L, with a 95 % confidence interval of 35 to 54 µg MITC/L. The NOEC was considered to be 24 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 98.9 % (according to certificate of analysis)
Test species:	Eastern Oyster (*Crassostrea virginica*)
Age of organisms:	Juveniles, approximately 31.4 – 39.5 mm valve height (umbo – distal edge)
Feeding:	Marine microalgae suspension provided continuously during holding at a nominal rate of approximately 2.9 x 109 cells/oyster/day and during the test at a nominal rate of approximately 5.8 x 109 cells/oyster/day
Type of test:	Flow-through toxicity test (19 volume replacements per day)
Applied concentrations:	Nominal test concentrations: 0 (dilution water control), 0 (solvent control 0.1 mL/L dimethylformamide), 7.5, 15, 30, 60 and 120 µg MITC/L
Number of organisms per group:	20 oysters for the control, for the solvent control and per treatment group
Time of exposure:	96 hours

	In-life dates: June 28th to July 2nd 2012
Test conditions:	temperature: 20.1 – 21.0 °C dissolved oxygen: 5.9 – 7.4 mg/L O_2 (≥ 74 % air saturation value) pH: 7.9 – 8.1 salinity: 20 ‰ photoperiod: 16 hours light and 8 hours dark light intensity: 305 lux
Test procedure:	Eastern Oyster (*Crassostrea virginica*) juveniles (approximately 31.4 – 39.5 mm valve height umbo – distal edge) were exposed by groups of 20 in a flow-through acute test for 96 hours to nominal concentrations of 0 (negative and solvent control), 7.5, 15, 30, 60 and 120 µg MITC/L (equivalent to the mean measured concentrations of 6.4, 13, 24, 44 and 92 µg MITC/L) in collected seawater. Test concentrations were determined in a range-finding test.
Test item analysis:	Samples were collected from each test chamber one day prior to the start of the test after conditioning the diluter for approximately 42 hours. Water samples were also collected from each test chamber at the beginning of the test and at 48 and 96 hours (± 1 hour) to measure concentrations of the test substance. The samples were collected from mid-depth, placed in glass vials, and processed immediately for analysis. A second set of samples was also collected at 96 hours and stored refrigerated for potential analysis if analytical confirmation was deemed necessary for a termination sample. The analytical method consisted of diluting the samples in acidified saltwater. Samples were extracted with diethyl ether. An aliquot of the diethyl ether extract was transferred to autosampler vials and submitted for analysis by gas chromatography with mass selective detection (GC/MS).
Observations:	Organisms were observed periodically to determine the number of mortalities in each treatment group. Oysters having open shells and not responding to gentle prodding were considered dead. The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were recorded. Observations were made approximately 18, 24, 48, 72 and 96 hours after test initiation. At the end of the test, the longest finger of new shell growth on each oyster was measured to the nearest 0.1 mm using calipers. Temperature was measured in each test chamber at the beginning and end of the test. Temperature was also monitored continuously in the negative control test chamber. Dissolved oxygen was measured in each test chamber at the beginning of the test, at approximately 24 hour intervals during the test, and at the end of the test. Measurements of pH were taken in each test chamber at the beginning of the test, at the approximate mid-point of the test (48 hours), and at the end of the test. Salinity was measured in each test chamber at the beginning and end of the test.
Statistical evaluation:	The shell deposition data from the negative control and solvent control were compared using a t-test. Since there were no significant differences between the control groups ($\alpha = 0.05$), growth inhibition was evaluated on the basis of the pooled control

data. The EC_{50} value, the concentration of test substance that inhibits shell deposition by 50 % relative to the pooled control, was calculated using linear interpolation. The shell deposition data were evaluated for normality and homogeneity of variance using the Chi-Square test and Levene's test, respectively. Since the data did not pass the assumptions of normality and homogeneity, a square root transformation was performed on the data. The transformed data passed the assumptions of normality using the Kolmogorov test, but transformations did not correct for homogeneity of variance. Additional data transformations did not correct the data for homogeneity of variance, therefore, the square root transformed data in the treatment groups were compared to the pooled control data using the Bonferroni t-test to identify any significant differences. The no-observed-effect concentration (NOEC) was determined from the statistical analysis of the data and an assessment of the concentration-response pattern. Statistical analyses were conducted using TOXSTAT® computer software.

Findings:

Analytical results:	Measured concentrations of the samples collected at the 0, 48 and 96 hour intervals ranged from 56.9 to 110 % of nominal. When measured concentrations of the samples collected during 0, 48 and 96 hour intervals were averaged, the mean measured test concentrations for this study were 6.4, 13, 24, 44 and 92 µg MITC/L, representing 85, 87, 80, 73 and 77 % of nominal concentrations, respectively.
Mortality and clinical signs of toxicity:	There were no mortalities among oysters in any treatment or control group during the test. All oysters in the negative and solvent control groups, and in the 6.4, 13, 24 and 44 µg MITC/L treatment groups appeared normal throughout the 96 hour exposure period. Oysters in the 92 µg MITC/L treatment group were normal in appearance, however, there were observations of reduced feeding (oysters closed) for this group. After 96 hours, the mean shell deposition in the negative and solvent control groups was 2.1 and 2.4 mm, respectively. When the shell deposition data for the negative control was compared with the solvent control, no statistically significant differences were found at the 95 % level of confidence. Therefore, the control data were pooled for comparison with the treatment groups. Mean shell deposition in the 6.4, 13, 24, 44 and 92 µg MITC/L treatment groups was 2.2, 1.8, 1.7, 0.8 and 0.04 mm, respectively. Dunnett's test indicated that there was a statistically significant decrease in shell deposition in the 44 and 92 µg MITC/L treatment groups in comparison to the pooled control ($p \leq 0.05$). For more details please refer to the following table.

Table B.2.9.2.2-33: New shell growth of the Eastern Oyster (*Crassostrea virginica*), exposed to MITC for 96 hours under flow-through test conditions

			New shell growth (mm)

Nominal concentration of test item (µg MITC/L)	Mean measured concentration of test item (µg MITC/L)	No. of *Crassostrea virginica*	Treatment mean (SD)	Percent Inhibition[1,2]
control	< LOQ	20	2.1 (0.95)	-
solvent control	< LOQ	20	2.4 (1.17)	-
pooled control	-	40	2.3 (1.06)	-
7.5	6.4	20	2.2 (1.10)	2.8
15	13	20	1.8 (1.09)	20
30	24	20	1.7 (0.99)	27
60	44	20	0.8 (1.06*)	64
120	92	20	0.04 (0.16*)	99

** Statistically significant difference from the pooled control using the Bonferroni t-test (α = 0.05)*
[1] Shell growth inhibition was calculated relative to the pooled control using Excel in full precision mode, manual calculations may differ slightly.
[2] 96-hour EC_{50} (95% confidence interval) = 42 µg MITC/L (35 – 54 µg MITC/L)

Assessment and conclusions:

Percent inhibition of Eastern Oyster (*Crassostrea virginica*) shell growth was calculated relative to the pooled control data. Inhibition of shell growth in the 6.4, 13, 24, 44 and 92 µg MITC/L treatment groups was 2.8 %, 20 %, 27 %, 64 % and 99 %, respectively. The 96 hour EC_{50} was calculated to be 42 µg MITC/L, with a 95 % confidence interval of 35 to 54 µg MITC/L. The NOEC was considered to be 24 µg MITC/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
EC_{50} (*Crassostrea virginica*, 96 h) = 0.042 mg MITC/L (mean measured)
NOEC (*Crassostrea virginica*, 96 h) = 0.024 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation and not relied upon in the geomean derivataion.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 % in control and 0 % in solvent control)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 5.9 – 7.4 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/63, for further details).

EC_{50} (*Crassostrea virginica*, 96 h, flow-through) = 42 µg MITC/L (based on mean measured concentrations)
NOEC (*Crassostrea virginica*, 96 h, flow-through) = 24 µg MITC/L (based on mean measured concentrations)
This study is considered acceptable. However, since the endpoint derived is based on shell growth and not on mortality, the study is not relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/10
Report author:	[redacted]
Report year:	2015
Report title:	Methyl isothiocyanate: Acute Toxicity to *Asellus aquaticus* in a 96-Hour Immobilization Test.
Report No.:	D89465
Document No.:	-
Guidelines followed in study:	US EPA Ecological Effects Test Guideline OPPTS 850.1020 "Gammarid acute toxicity test"
Deviations from current test guideline:	Deviations from US EPA OPPTS 850.1020: Test species: Asellus aquaticus (proposed: *Gammarus fasciatus*, *Gammarus pseudolimnaeus* or *Gammarus lacustris*)
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a semi-static acute toxicity test, freshwater crustacean (*Asellus aquaticus*) were exposed individually in 250 mL vessels filled with reconstituted test water. The organisms were exposed for 96 hours, with medium renewal after 48 hours, to nominal concentrations of 0 (negative control), 0.010, 0.022, 0.046, 0.10 and 0.22 mg MITC/L. The concentrations as % nominal found in the fresh test samples ranged from 79 % to 92 % (day 0) and from 61 % to 83 % (day 2). The concentrations as % nominal found in the old test samples ranged from 64 % to 74 % (day 2/48 h) and from 78 % to 80 % (day 4/48 h). In the control and at the test concentrations up to and including 0.035 mg MITC/L, no mortality or visible abnormalities were observed in the test organisms during the test period of 96 hours. At the higher test concentrations of 0.075 and 0.17 mg MITC/L (mean measured) the mortality was 30 % and 85 %, respectively.
The test item had acute toxic effects on *Asellus aquaticus*. The 96 hour LC_{50} was determined based on mean measured concentrations to be 0.11 mg MITC/L with 95 % confidence limits of 0.09 and 0.13 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: A0337662, chemical purity: 98.9 % (according to certificate of analysis)
Test species:	crustacean (*Asellus aquaticus*)
Age of organisms:	Crustaceans of approximately 1.0 cm
Type of test:	Semi-static toxicity test (medium renewal after 48 hours)
Applied concentrations:	Nominal test concentrations:

	0 (control), 0.010, 0.022, 0.046, 0.10 and 0.22 mg MITC/L
Number of organisms per group:	20 organisms for the control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 19.0 °C dissolved oxygen: 8.1 – 8.5 mg/L O_2 pH: 7.2 – 7.4 hardness: 1.25 mmol/L $CaCO_3$ alkalinity: 0.4 mmol/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 280 – 450 lux
Test procedure:	Twenty freshwater crustacean (*Asellus aquaticus*) (of approximately 1.0 cm) were exposed individually in 250 mL vessels in a semi-static acute test for 96 hours, with medium renewal after 48 hours to nominal concentrations of 0 (negative control), 0.010, 0.022, 0.046, 0.10 and 0.22 mg MITC/L. Test concentrations were determined in a range-finding test and on results of a first main test, which was repeated since the validity criteria were not met.
Test item analysis:	For the determination of the actual test item concentrations, duplicate samples were taken from each treatment at the start and end of each test medium renewal period. For the stability samples, the contents of the respective replicates were combined prior to sampling. All samples were stored deep-frozen (at about -20 °C) immediately after sampling. Based on pre-experiments for investigation of the storage stability, the test item was found to be stable in the test water under these storage conditions. The concentrations of the test item were analyzed in one of the duplicate test media samples from the nominal concentrations of 0.046 to 0.22 mg MITC/L and from the control from all sampling times. The samples of the nominal test concentrations of 0.022 and 0.010 mg MITC/L were not analysed since the concentrations were below the 96 hour NOEC determined in this test and, thus, were not relevant for the interpretation of the biological results. The mean measured concentration during the test period was calculated as the arithmetic mean of all measurements per test concentration (freshly prepared and aged media) during the test period. The samples were analysed by gas chromatography with flame ionization detection (GC-FPD).
Observations:	The mortality of the *Asellus aquaticus* was determined by visual inspection after 24, 48, 72 and 96 hours of exposure. Those test animals which did not react after gentle prodding were considered to be dead. At the start and end of each test medium renewal period, the pH values, dissolved oxygen concentrations and water temperature were determined at each treatment. The appearance of the test media was visually recorded at the same time.
Statistical evaluation:	The 72 and 96 hour EC_{50} were calculated by Weibull Analysis. The 24 and 48 hour EC_{50} of the test item could not be calculated, because none of the responses exceeded 50 %. The lowest nominal concentrations of 0.022 and 0.010 mg MITC/L were not taken into account at the statistical calculations because

they were below the determined 96 hour NOEC and, thus, not analysed. The NOEC, EC_0 and EC_{100} were determined directly from the raw data. All biological results and statistical calculations are based on mean measured test item concentrations.

Findings:

Analytical results: The concentrations as % nominal found in the fresh test samples ranged from 79 to 92 % (day 0) and from 61 to 83 % (day 2). The concentrations as % nominal found in the old test samples ranged from 64 to 74 % (day 2/48 h) and from 78 to 80 % (day 4/48 h). No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the entire test duration.

Mortality and clinical signs of toxicity: In the control and at the test concentrations up to and including 0.035 mg MITC/L, no mortality or visible abnormalities were observed at the test organisms during the test period of 96 hours. At the higher test concentrations of 0.075 and 0.17 mg MITC/L, the mortality was 30 % and 85 %, respectively. For further details please refer to the following table.

Table B.2.9.2.2-34: Mortality of the freshwater crustacean (*Asellus aquaticus*), exposed to MITC for 96 hours under semi-static test conditions

Nominal concentration of test item (mg MITC/L)	Mean measured concentration of test item (mg MITC/L)	No. of *Asellus aquatic-cus*	Mortality at							
			24 hours		48 hours		72 hours		96 hours	
			No.	Inhibi-tion [%]	No.	Inhibi-tion [%]	No.	Inhibi-tion [%]	No.	Inhibi-tion [%]
control	--	20	0	--	0	--	0	--	0	--
0.010	n.a.	20	0	0	0	0	0	0	0	0
0.022	n.a.	20	0	0	0	0	0	0	0	0
0.046	0.035	20	0	0	0	0	0	0	0	0
0.10	0.075	20	0	0	5	25	6	30	6	30
0.22	0.17	20	2	10	4	20	10	50	17	85

n.a.= not analysed since below NOEC

Assessment and conclusions:

The test item had acute toxic effects on *Asellus aquaticus*. The 96 hour LC_{50} was determined based on mean measured concentrations to be 0.11 mg MITC/L with 95 % confidence limits of 0.09 and 0.13 mg MITC/L. The 96 NOEC was determined to be 0.035 mg MITC/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Asellus aquaticus*, 96 h) = 0.11 mg MITC/L (mean measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.

Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.1 – 8.5 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/55, for further details).
There is uncertainty on the lower measured test concentrations, but since used in a SSD calculation, the endpoint is considered acceptable, though slight deficiencies.
LC_{50} (*Asellus aquaticus*, 96 h, semi-static) = 0.11 mg MITC/L (based on mean measured concentrations)
NOEC (*Asellus aquaticus*, 96 h, semi-static) = 0.035 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/11
Report author:	[redacted]
Report year:	2014a
Report title:	Methyl isothiocyanate: Acute Toxicity to *Thamnocephalus platyurus* in a 24-Hour Immobilization Test.
Report No.:	D85360
Document No.:	-
Guidelines followed in study:	The test method followed the standard operational procedure of the commercial test kit used and are based on the OECD Guideline for Testing of Chemicals, No. 202 (2004) and the OPPTS Guideline No. 850.1010
Deviations from current test guideline:	Deviations from current OECD Guideline 202 (2004): Test species: *Thamnocephalus platyurus* (recommendation: *Daphnia magna*) Test temperature: 24 °C (recommendation: 18 – 22 °C) Test system: static (recommendation: flow-through or semi-static)
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a static acute toxicity test, freshly hatched shrimps (*Thamnocephalus platyurus*) were exposed by groups of 10 organisms in 3 replicates per treatment 24 hours to nominal concentrations of 0 (negative control), 0.13, 0.32, 0.80, 2.0 and 5.0 mg Methyl Isothiocyanate (MITC)/L in reconstituted test water. The measured concentrations of Methyl isothiocyanate in the test media of the test concentrations of nominal 5.0, 2.0 and 0.80 mg/L were between 98 % and 113 % of the nominal values at the start and the end of the test. The measured concentrations for the nominal concentrations of 0.80, 2.0 and 5.0 mg MITC/L were 0.787, 2.00 and 5.65 mg MITC/L after 0 hours and 0.821, 2.08 and 5.38 mg MITC/L after 24 hours, respectively. The biological results were related to the nominal concentrations of the test item. During the 24 hours of the test, no immobilised test organisms were determined in the control and up to and including the test item concentration of 0.80 mg MITC/L. At the next higher concentration of 2.0 mg MITC/L, the immobilization was 47 %. At the highest test concentration of 5.0 mg MITC/L all test organisms were found to be immobile at the observation after 24 hours.
MITC had acute toxic effects on *Thamnocephalus platyurus*. The 24 hour EC_{50} was determined to be 2.0 mg MITC/L with 95 % confidence limits of 1.7 and 2.4 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: A0337662, chemical purity: 98.9 % (according to certificate of analysis)
Test species:	crustacean (*Thamnocephalus platyurus*)
Age of organisms:	Freshly hatched shrimps, about 4 hours old (II-III larvae at test initiation)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.13, 0.32, 0.80, 2.0 and 5.0 mg MITC/L
Number of organisms per group:	10 organisms per replicate, 3 replicates for the control and per treatment group
Time of exposure:	24 hours
Test conditions:	temperature: 24.0 °C dissolved oxygen: 8.5 – 8.9 mg/L O_2 pH: 8.0 hardness: 2.5 mmol/L $CaCO_3$ alkalinity: 0.8 mmol/L $CaCO_3$ photoperiod: 24 hours dark
Test procedure:	Freshly hatched shrimps (*Thamnocephalus platyurus*) (of approximately 4 hours old) were exposed by groups of 10 organisms in 3 replicates per treatment in a static acute test for 24 hours to nominal concentrations of 0 (negative control), 0.13, 0.32, 0.80, 2.0 and 5.0 mg MITC/L. The measured concentrations of MITC in the test media of the test concentrations of nominal 5.0, 2.0 and 0.80 mg MITC/L were between 98 and 113 % of the nominal values at the start and the end of the test. Test concentrations were determined in a range-finding test.
Test item analysis:	One 10 mL and (as a backup) one 100 mL samples were taken from all test concentrations and the control at the start and at the end of the test (after 24 hours). For the 24 hour stability samples, additional flasks with adequate volumes of the freshly prepared test media of all test concentrations and the control were incubated during the test period under the same conditions as in the actual test (but without test organisms). Sampling from the test itself was not possible because the test medium volume was too small for

analytical requirements. To all 10 mL samples, 3 mL (tert-Butyl)methylether were added to stabilise the samples during the storage period. The 100 mL samples were not treated. Thereafter, all samples were deep-frozen (at about -20 °C).
The concentrations of the test item were analysed in the 10 mL test media samples from the nominal concentrations of 5.0 to 0.80 mg/L from both sampling times (0 and 24 hours). The samples of the nominal test concentrations of 0.32 and 0.13 mg/L were not analysed since these concentrations were below the 24 hour NOEC determined in this test and, thus, were not relevant for the interpretation of the biological results. From the control, one of the duplicate samples was analysed per sampling time.
The test item concentration in the test samples was determined by gas chromatography (GC) using external calibration.
The test item gave a chromatographic profile consisting of a single peak. To demonstrate the validity of the method, untreated test media was spiked with the test item. Five spiked recovery samples were freshly prepared per concentration level, subjected to the same treatment as a test sample but without storage and subsequently analyzed. In addition, test water without the test item was analyzed after sample preparation (analytical blank).

Observations: The immobility of *Thamnocephalus platyurus* was determined by visual inspection after 24 hours of exposure. Those test organisms not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. At the start and end of the test, the pH values, dissolved oxygen concentrations and water temperature were determined at each treatment. The appearance of the test media was visually recorded at the start of the test and after 24 hours.

Statistical evaluation: The NOEC, EC_0 and EC_{100} were determined directly from the raw data. The 24 hour EC_{50} and the 95 % confidence limits were calculated by probit analysis using linear maximum likelihood regression.

Findings:

Analytical results: The measured concentrations of Methyl isothiocyanate in the test media of the test concentrations of nominal 5.0, 2.0 and 0.80 mg/L were between 98 and 113 % of the nominal values at the start and the end of the test. Thus, the correct dosage of the test item was confirmed and the test item was stable in the test media over the test period of 24 hours. The biological results were related to the nominal concentrations of the test item.

Immobilisation: During the 24 hours of the test, no immobilised test organisms were determined in the control and up to and including the test item concentration of 0.80 mg MITC/L. At the next higher concentration of 2.0 mg MITC/L, the immobilisation was 47 %. At the highest test concentration of 5.0 mg MITC/L all test organisms were found to be immobile at the observation after 24 hours. For further details please refer to the following table.

Table B.2.9.2.2-35: Immobilisation of the shrimp (*Thamnocephalus platyurus*), exposed to MITC for 24 hours under static test conditions

Nominal concentration of test item (mg MITC/L)	Measured concentration of test item (mg MITC/L)		No. of *Thamnocephalus platyurus*	Immobilisation at 24 hours	
	after 0 hours	after 24 hours		No.	Percent [%]
control	< LOQ	< LOQ	30	0	0
0.13	n.d.	n.d.	30	0	0
0.32	n.d.	n.d.	30	0	0
0.80	0.787	0.821	30	0	0
2.0	2.00	2.08	30	14	47
5.0	5.65	5.38	30	30	100

n.d. – not determined

Assessment and conclusions:

Methyl Isothiocyanate (MITC) had acute toxic effects on *Thamnocephalus platyurus*. The 24 hour EC_{50} was determined to be 2.0 mg MITC/L with 95 % confidence limits of 1.7 and 2.4 mg MITC/L. The 24 hour EC_0 and the 24 hour NOEC were 0.80 mg MITC/L. The 24 hour EC_{100} was 5.0 mg MITC/L.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

LC_{50} (*Thamnocephalus platyurus*, 24 h) = 2.0 mg MITC/L (nominal)

Analytical method:

Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.5 – 8.9 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.

Therefore, this study is considered acceptable.

The analytical method used could be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/53, for further details).

LC_{50} (*Thamnocephalus platyurus*, 24 h, static) = 2.0 mg MITC/L (based on nominal concentrations)

NOEC (*Thamnocephalus platyurus, 24 h, static*) = 0.80 mg MITC/L (based on nominal concentrations)

This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/12
Report author:	[redacted]
Report year:	2014b
Report title:	Methyl isothiocyanate: Acute Toxicity to *Brachionus calyciflorus* in a 24-Hour Immobilization Test.
Report No.:	D85371
Document No.:	-
Guidelines followed in study:	Test method was based on the recommendations of international testing guidelines such as e.g. OECD 202 and US EPA OPPTS 850.1010 and follows the standard operational procedure of the commercial test kit used.
Deviations from current test guideline:	Deviations from current OECD Guideline 202 (2004): Test species: *Brachionus calyciflorus* (recommendation: *Daphnia magna*) Test system: static (recommendation: flow-through or semi-static)
Previous evaluation:	No, not previously submitted at EU level Submitted in the framework of national authorisation
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF

Study Summary:

In a static acute toxicity test, rotifier larvae (*Brachionus calyciflorus*) were exposed by groups of 30 organisms divided into 6 replicates per treatment for 24 hours to nominal concentrations of 0 (negative control), 0.10, 0.20, 0.40, 0.80 and 1.6 mg Methyl Isothiocyanate (MITC)/L in reconstituted test water. The mean measured concentrations after 24 hours for the nominal concentrations of 0.80 and 1.60 mg MITC/L were 0.56 and 1.20 mg MITC/L, respectively.
In the control and up to and including the test concentration of 1.2 mg MITC/L (mean measured), no immobilised test organisms were observed during the test period of 24 hours.
The test item Methyl isothiocyanate had no acute toxic effects on *Brachionus calyciflorus* up to the test concentration of 1.2 mg MITC/L under the conditions of the test. The 24 hour EC_{50} and EC_{100} were calculated to be > 1.2 mg MITC/L. The 24 hour EC_0 and NOEC were determined to be 1.2 mg MITC/L

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: A0337662, chemical purity: 98.9 % (according to certificate of analysis)
Test species:	rotifers (*Brachionus calyciflorus*)
Age of organisms:	Freshly hatched larvae
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.10, 0.20, 0.40, 0.80 and 1.6 mg MITC/L
Number of organisms per group:	5 organisms per replicate, 6 replicates for the control and per treatment group
Time of exposure:	24 hours
Test conditions:	temperature: 21.0 °C dissolved oxygen: 8.1 – 8.9 mg/L O_2 pH: 8.0

	hardness: 2.5 mmol/L $CaCO_3$ alkalinity: 0.8 mmol/L $CaCO_3$ photoperiod: 24 hours dark
Test procedure:	Freshly hatched rotifer larvae (*Brachionus calyciflorus*) were exposed by groups of 30 organisms divided into 6 replicates per treatment in a static acute test for 24 hours to nominal concentrations of 0 (negative control), 0.10, 0.20, 0.40, 0.80 and 1.6 mg MITC/L in reconstituted test water. The mean measured concentrations after 24 hours for the nominal concentrations of 0.80 and 1.60 mg MITC/L were 0.56 and 1.20 mg MITC/L, respectively. Test concentrations were determined in a range-finding test.
Test item analysis:	One 10 mL and (as a backup) duplicate 100 mL samples were taken from all test concentrations and the control at the start and at the end of the test (after 24 hours). For the 24 hour stability samples, additional flasks with adequate volumes of the freshly prepared test media of all test concentrations and the control were incubated during the test period under the same conditions as in the actual test (but without test organisms). Sampling from the test itself was not possible because the test medium volume was too small for analytical requirements. To all 10 mL samples, 3 mL (*tert*-Butyl)methylether were added to stabilise the samples during the storage period. The 100 mL samples were not treated. Thereafter, all samples were deep-frozen (at about -20 °C). The concentrations of the test item were analysed in the test media samples from the nominal concentrations of 1.6 ad 0.80 mg/L from both sampling times (0 and 24 hours). The samples of the nominal test concentrations of 0.40 to 0.10 mg/L were not analysed since these concentrations were below the 24 hour NOEC determined in this test and, thus, were not relevant for the interpretation of the biological results. From the control, one of the duplicate samples was analysed per sampling time. The test item concentration in the test samples was determined by gas chromatography (GC) using external calibration. The test item gave a chromatographic profile consisting of a single peak. To demonstrate the validity of the method, untreated test media was spiked with the test item. Five spiked recovery samples were freshly prepared per concentration level, subjected to the same treatment as a test sample but without storage and subsequently analysed. In addition, test water without the test item was analysed after sample preparation (analytical blank).
Observations:	The immobility of the *Brachionus calyciflorus* was determined by visual inspection after 24 hours of exposure. Those test organisms not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. At the start and end of the test, the pH values, dissolved oxygen concentrations and water temperature were determined at each treatment. The appearance of the test media was visually recorded at the start of the test and after 24 hours.
Statistical evaluation:	No statistical methods were employed as no effects were observed in the organisms up to and including the highest tested concentration.

Findings:

Analytical results: The measured concentrations of MITC in the test media of the test concentrations of nominal 0.80 and 1.6 mg MITC/L were 73 and 80 % of the nominal values at the start of the test. At the end of the test, 67 and 74 % of the nominal were found. The mean measured test item concentrations (calculated as the geometric means of the concentrations measured at the start and end of the test) were 0.56 mg MITC/L (nominal 0.80 mg MITC/L) and 1.2 mg MITC/L (nominal 1.6 mg MITC/L). The biological results were related to the mean measured test item concentrations.

Immobilisation: In the control and up to and including the test concentration of 1.2 mg MITC/L (mean measured), no immobilised test organisms were observed during the test period of 24 hours.

Table B.2.9.2.2-36: Immobilisation of the rotifer (*Brachionus calyciflorus*), exposed to MITC for 24 hours under static test conditions

Nominal concentration of test item (mg MITC/L)	Mean measured concentration of test item (mg MITC/L)	No. of *Brachionus calyciflorus*	Immobilization at 24 hours	
			No.	Percent [%]
control	---	30	0	0
0.10	n.d.	30	0	0
0.20	n.d.	30	0	0
0.40	n.d.	30	0	0
0.80	0.56	30	0	0
1.60	1.20	30	0	0

n.d. – not determined

Assessment and conclusions:

The test item Methyl Isothiocyanate had no acute toxic effects on *Brachionus calyciflorus* up to the mean measured test concentration of 1.2 mg MITC/L under the conditions of the test. The 24 hour EC_{50} and EC_{100} were calculated to be > 1.2 mg MITC/L (mean measured). The 24 hour EC_0 and NOEC were determined to be 1.2 mg MITC/L (mean measured).

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

LC_{50} (*Brachionus calyciflorus*, 24 h) > 1.2 mg MITC/L (mean measured)

Analytical method:

Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation, but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose as taken into account for the geomean derivation.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 8.1 – 8.9 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/54, for further details).
LC_{50} (*Brachionus calyciflorus*, 24 h, static) > 1.2 mg MITC/L (based on mean measured concentrations)
NOEC (*Brachionus calyciflorus*, 24 h, static) = 1.2 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/13
Report author:	[redacted]
Report year:	2019a
Report title:	*Lumbriculus variegatus*, acute toxicity test (96 hours) – Semi-static test conditions. Test item: Methyl Isothiocyanate (MITC)
Report No.:	TAM-003/4-22/U
Document No.:	-
Guidelines followed in study:	OECD 225 (Sediment-Water *Lumbriculus* Toxicity Test using Spiked Sediment) OECD 202 (*Daphnia magna*, Acute Immobilisation Test)
Deviations from current test guideline:	Deviations from current OECD Guideline 225 (2007): The test was conducted over 96 hours and water only. As endpoint, the immobilisation of test organisms was investigated. Deviations from current OECD Guideline 202 (2004): Test species: *Lumbriculus variegatus* (recommendation: *Daphnia magna*)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

A study was performed to investigate the influence of the test item Methyl Isothiocyanate (MITC) on the mobility of freshwater oligochaete *Lumbriculus variegatus*. The test was conducted under semi-static conditions over a period of 96 hours with one media exchange after 48 hours.

The test organisms were placed in water containing the test item in nominal concentrations of 0.1, 0.18, 0.32, 0.58 and 1.05 mg MITC/L. A control with test medium only and a solvent control containing the solubilising agent ethanol were run in parallel. Effects on immobilisation were determined daily.
The concentrations of the test item in the media were confirmed by measurements at beginning of the test, at media renewal after 48 hours (aged and fresh test solutions) and at test end by GC-MS (LOQ = 0.09 mg/L). Concentrations of freshly prepared test media showed recovery rates between 99.9 % and 120 % of nominal concentrations. Concentrations in aged test media were between 55.1 % to 115 % of initial concentrations and showed recovery rates between 66.0 % and 126 % of nominal concentrations. The measured concentrations were not within a range of 80 – 120 % of nominal concentrations, therefore, the evaluation was based on the geometric mean measured concentrations of 0.104, 0.181, 0.345, 0.592 and 1.05 mg MITC/L (100 – 108 % of nominal concentrations).
Since no statistically significant effect between the control and solvent control could be observed, treatments were compared against the solvent control.
The test item had a statistically significant effect on the immobilisation of the test organisms in a concentration dependent manner. The mortality was ≤ 10 % in the control and in the solvent control. The dissolved oxygen concentration in the overlying water was not below 30 % of air saturation value (ASV) in both controls throughout the test. The pH of the overlying water between 6 and 9 throughout the test. Thus, according to the OECD guideline 225 and 202 the test was considered valid.
The test item MITC was acutely toxic to the test organism *Lumbriculus variegatus* in a 96 hour acute toxicity test. The LC_{50} for immobilisation of the test organisms was calculated to be 0.315 mg MITC/L. The NOEC was determined to be 0.181 mg MITC/L and the LOEC was 0.345 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBH5869, chemical purity: ≥ 96.5 % (according to certificate of analysis)
Test species:	Freshwater oligochaete (*Lumbriculus variegatus*)
Age of organisms:	Intact complete worms, which were actively swimming or crawling upon a gentle mechanical stimulus; the organisms were in a similar length and were in a similar physiological state
Type of test:	Semi-static toxicity test (medium renewal after 48 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control 100 µL/L or 0.01 % (v/v) ethanol), 0.1, 0.18, 0.32, 0.58 and 1.05 mg MITC/L
Number of organisms per group:	5 organisms per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 21.8 – 22.1 °C dissolved oxygen: 4.69 – 8.03 mg/L O_2 (59.1 – 96.9 % air saturation value) in the controls; 2.18 – 8.08 mg/L O_2 (26 – 97 % air saturation value) in the treatments pH: 6.20 – 7.00 hardness: 90 – 300 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 810 – 878 lux
Test procedure:	*Lumbriculus variegatus* were exposed to the test substance added to water at a range of five concentrations for 96 hours under semi-static conditions with renewal of test medium once after 48 hours. The total number of immobile individuals was assessed daily. The test was performed in four replicates per concentration with five specimens per replicate (according to OECD guideline 202).

	Reconstituted (synthetic) water was used as test medium according to OECD guideline 225. The concentrations to be tested in the definitive test were selected based on the results from the range-finding test: control, solvent control, 0.1, 0.18, 0.32, 0.58 and 1.05 mg MITC/L. The control consisted of test medium only. In addition, a solvent control containing the solubilising agent ethanol was applied at the level used in the treatments (100 µL/L or 0.01 % (v/v)).
Test item analysis:	The concentrations of MITC in the water phase were measured by chemical analysis. Samples of freshly prepared test media were taken from all five test solutions and controls at the beginning of the test and at media renewal after 48 hours prior to distribution to the test vessels. Samples of aged test media were taken directly from representative or pooled replicates per test concentration and controls at media renewal and at test end. The test item was analysed using GC-MS. If analysis could not be applied immediately after sampling, samples were stored under circumstances to maintain the test item stable.
Observations:	The number of immobile worms was visually determined daily. Any abnormalities in appearance and behaviour were recorded, if occurred. Oxygen concentration, pH value and temperature of the test solutions was checked at test start, at media renewal and at test end.
Statistical evaluation:	Where the test results show inhibition around 50 % they were statistically analysed to determine an LC_{50} value together with 95 % confidence intervals using Probit-analysis assuming log-normal distribution of the values. The NOEC and LOEC values were determined using the step-down Cochran-Armitage test procedure. The computer program ToxRat was used for statistical evaluations.

Findings:

Analytical results:	At test initiation, concentrations of 0.107, 0.180, 0.335, 0.635 and 1.13 mg MITC/L (99.9 – 110 % of nominal) were measured. At media renewal after 48 hours, measured concentrations of aged samples were at 0.105, 0.189, 0.344, 0.729 and 0.985 mg MITC/L (93.8 – 126 % of nominal; 87.3 – 115 % of initial concentrations). Fresh samples at 48 hours showed measured concentrations of 0.107, 0.182, 0.346, 0.694 and 1.18 mg MITC/L (101 – 120 % of nominal concentrations). At test end, concentrations of 0.098, 0.175, 0.353 and 0.383 mg MITC/L (66.0 – 111 % of nominal; 55.1 – 102 % of initial concentrations). The measured concentrations of freshly prepared test solutions were not within a range of 80 – 120 % of the nominal concentrations, therefore, the evaluation was based on the geometric mean measured concentrations of the test item, which were 0.104, 0.181, 0.345, 0.592 and 1.05 mg MITC/L (100 – 108 % of nominal concentrations).

Immobilisation, physical/pathological symptoms and changes in behaviour:	In the control, solvent control and up to and including the test concentration of 0.181 mg MITC/L (mean measured) no immobilised test organisms were observed during the test period of 96 hours. At the beginning of the test (24 – 48 hours) test organisms in the controls and low test concentrations of 0.104 and 0.181 mg MITC/L were building kind of balls while organisms in the treatments containing MITC in higher concentrations (0.345, 0.592 and 1.05 mg MITC/L) were lying separately and elongated. After 48 hours, test organisms in all test concentrations were lying separately and elongated while organisms in the controls were building balls further on. The test item MITC, had a statistically significant effect on the immobility of the test organisms in a concentration dependent manner. The LC_{50} for immobility was calculated to be 0.315 mg MITC/L and the NOEC was determined at 0.181 mg MITC/L. All results were calculated based on geometric mean measured concentrations.

Table B.2.9.2.2-37: Results of the chemical analysis of the test item

Nominal concentration	Test initiation (0 hours)		Media renewal (48 hours)		
	Fresh test solutions		Aged test solutions		
	Measured concentration	% of nominal	Measured concentration	% of nominal	% of initial
[mg MITC/L]	[mg MITC/L]	[%]	[mg MITC/L]	[%]	[%]
control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.10	0.107	107	0.105	105	98.0
0.18	0.180	99.9	0.189	105	105
0.32	0.335	105	0.344	107	103
0.58	0.635	110	0.729	126	115
1.05	1.129	108	0.985	93.8	87.3
Nominal concentration	Media renewal (48 hours)		Test termination (96 hours)		
	Fresh test solutions		Aged test solutions		
	Measured concentration	% of nominal	Measured concentration	% of nominal	% of initial
[mg MITC/L]	[mg MITC/L]	[%]	[mg MITC/L]	[%]	[%]
control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.10	0.107	107	0.098	97.6	91.4
0.18	0.182	101	0.175	97.0	95.9
0.32	0.346	108	0.353	111	102
0.58	0.694	120	0.383	66.0	55.1
1.05	1.179	112	*	*	*

LOQ: 0.09 mg MITC/L

**: all test organisms died during the first 48 hours, therefore no organisms were exposed after medium renewal and, thus, no aged samples was available at test termination.*

Table B.2.9.2.2-38: Geometric mean measured concentrations of the test item

Nominal concentration	Geometric mean measured concentrations

[mg MITC/L]	[mg MITC/L]	[%]
control	< LOQ	-
solvent Control	< LOQ	-
0.10	0.104	104
0.18	0.181	101
0.32	0.345	108
0.58	0.592	102
1.05	1.05	100

LOQ: 0.09 mg MITC/L

Table B.2.9.2.2-39: Immobilisation of the freshwater oligochaete (Lumbriculus variegatus), exposed to MITC for 96 hours under semi-static test conditions

Geometric mean measured concentration [mg MITC/L]	Total *Lumbriculus variegatus* introduced	Mobile	Immobile	% Immobility
solvent control	20	20	0	0.0
0.104	20	20	0	0.0 (-)
0.181	20	20	0	0.0 (-)
0.345	20	4	16	80.0 (+)
0.592	20	0	20	100 (+)
1.054	20	0	20	100 (+)

(+): significant; (-): non-significant;
Step-down Cochran-Armitage test procedure, significance level was 0.050, one-sided greater.

The response curve for immobilisation at 96 hours of *Lumbriculus variegatus* is shown in the following figure.

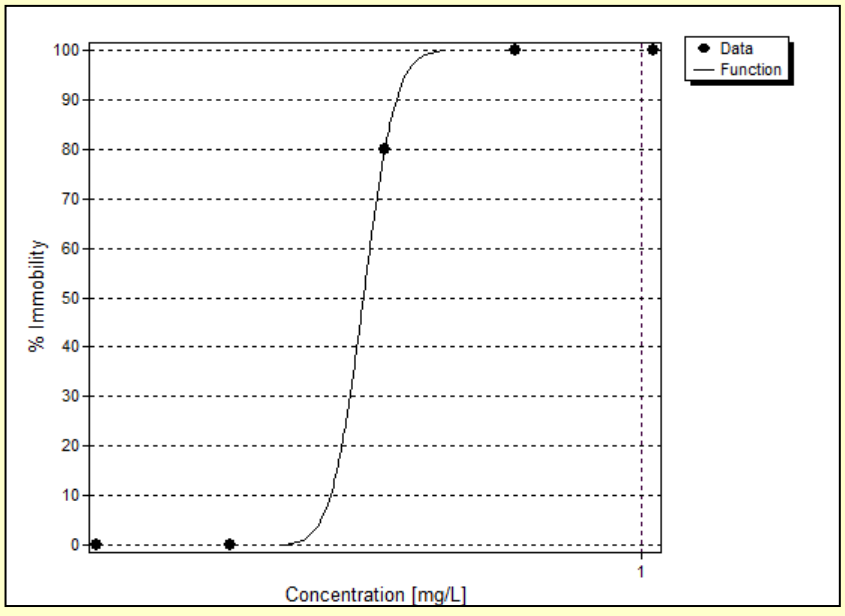


Figure B.2.9.2.2-1: Concentration-effect curve showing the influence of the test item on the immobility of the introduced test organisms as observed after 96 hours

In order to confirm the sensitivity of the test species *Lumbriculus variegatus*, acute immobilisation tests over 96 hours with the reference substance Pentachlorphenol (PCP) are performed at regular intervals, as

proposed by OECD guideline 225. The results of the latest reference study (July 2019) are in the range of toxicity as reported in OECD guideline 225:

EC_{50} (*Lumbriculus variegatus*, 96 hours) for immobilisation = 241.7 µg/L
(95 % confidence limits : 206 – 283 µg/L)

Assessment and conclusions:

The test item Methyl Isothiocyanate had a statistically significant effect on immobility of the test organism *Lumbriculus variegatus* in a concentration dependent manner.
The LC_{50} for immobility was calculated to be 0.315 mg MITC/L and the NOEC was determined at 0.181 mg MITC/L. All results were calculated based on geometric mean measured concentrations.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Lumbriculus variegatus*, 96 h) = 0.315 mg MITC/L (mean measured)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The test item Methyl Isothiocyanate (MITC) had a statistically significant effect on immobility of the test organism *Lumbriculus variegatus* in a concentration dependent manner.
The LC_{50} for immobility was calculated to be 0.315 mg MITC/L.
The NOEC was determined at 0.181 mg MITC/L.
All results were calculated based on geometric mean measured MITC concentrations.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were not all met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 4.69 – 8.03 mg/L O_2 (59.1 – 96.9 % air saturation value) in the controls; 2.18 – 8.08 mg/L O_2 (26 – 97 % air saturation value) in the treatments)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
The validity criteria of OECD Guideline 225 were not all met:

- the average number of living worms per replicate in the controls should have increased by a factor of at least 1.8 at the end of the exposure compared to the number of worms per replicate at the start of exposure (not reported in the study)
- the pH of the overlying water should be between 6 and 9 throughout the test (measured: 6.20 – 7.00)
- the oxygen concentration in the overlying water should not be below 30 % of air saturation value (ASV) at test temperature during the test (measured: 4.69 – 8.03 mg/L O_2 (59.1 – 96.9 % air saturation value) in the controls; 2.18 – 8.08 mg/L O_2 (26 – 97 % air saturation value) in the treatments)

Despite that the measured oxygen concentration was at certain moment slightly below the recommended 30 % of air saturation value, this study is considered acceptable.

(The measured oxygen concentration was 2.56, 2.18, 2.62 and 2.21 mg/L O_2 in the test item concentrations of 0.104, 0181, 0.345 and 1.054 mg MITC/L in the aged media after the first 48 hours of exposure. The measured oxygen concentration was 2.98, 2.69 and 2.75 mg/L O_2 in the test item concentrations of 0.104, 0.181 and 0.345 mg MITC/L in the aged media after the second part of 48 hours of exposure (at test termination 96 hours). Since no immobilisation occurred in the 0.104 and 0.181 mg MITC/L test concentrations, the low oxygen concentration had no impact on the endpoint. Also, since the endpoint LC_{50} = 0.315 mg MITC/L, the low oxygen concentration at 0.345 and 1.054 mg MITC/L did not impact the validity of the study.)
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/75 (Taminco) and KCA 4.1.2/26 (Lainco), for further details).
LC_{50} (*Lumbriculus variegatus*, 96 h, semi-static) = 0.315 mg MITC/L (based on mean measured concentrations)
NOEC (*Lumbriculus variegatus*, 96 h, semi-static) = 0.181 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/14
Report author:	[redacted]
Report year:	2019b
Report title:	*Potamopyrgus antipodarum*, acute toxicity test (96 hours) – Semi-static test conditions. Test item: Methyl Isothiocyanate (MITC)
Report No.:	TAM-003/4-22/W
Document No.:	-
Guidelines followed in study:	OECD 242 (*Potamopyrgus antipodarum* Reproduction Test) OECD 202 (*Daphnia magna*, Acute Immobilisation Test)
Deviations from current test guideline:	Deviations from current OECD Guideline 242 (2016): The test was conducted over 96 hours only. As endpoint, the immobilisation of test organisms was investigated. Deviations from current OECD Guideline 202 (2004): Test species: *Potamopyrgus antipodarum* (recommendation: *Daphnia magna*)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

A study was performed to investigate the influence of the test item Methyl Isothiocyanate (MITC) on the mobility of snails of the species *Potamopyrgus antipodarum*. The test was conducted under semi-static conditions over a period of 96 hours with one media exchange after 48 hours.

The snails were placed in water containing the test item in nominal concentrations of 0.01, 0.03, 0.10, 0.32 and 1.00 mg MITC/L. A control with test medium only and a solvent control containing the solubilising agent ethanol were run in parallel. Effects on immobilisation were determined daily.
The concentrations of the test item in the media were confirmed by measurements at beginning of the test, before and after media renewal after 48 hours and at test end by GC-MS (LOQ = 0.005 mg/L). Concentrations of freshly prepared test media showed recovery rates between 97.7 % and 116 % of nominal concentrations. Concentrations in aged test media remained stable with 95.4 % to 113 % of initial concentrations and showed recovery rates between 101 % and 121 % of nominal concentrations. The measured concentrations were mainly within a range of 80 – 120 % of nominal concentrations. However, at the highest test concentration the measured concentration deviated by more than 20 % of the nominal concentration. The test concentrations remained stable during each of the incubation periods of 48 hours. The evaluation was based on the geometric mean measured concentrations of 0.010, 0.032, 0.112, 0.365 and 1.133 mg MITC/L (105 – 1148 % of nominal concentrations).
Since no statistically significant effect between the control and solvent control could be observed, treatments were compared against the solvent control.
The test item had a statistically significant effect on the immobilisation of the snails in a concentration dependent manner. The mortality was ≤ 20 % in the control and in the solvent control. The dissolved oxygen concentration was > 60 % of the air saturation value (ASV) in both control and exposure groups throughout the test. The mean water temperature was in the range of 16 ± 1.5 °C given in the OECD guideline 242 for chronic testing of *Potamopyrgus antipodarum*. Thus, the test was considered valid.
The test item MITC was acutely toxic to the test organism *Potamopyrgus antipodarum* in a 96 hour acute toxicity test. The LC_{50} for immobilisation of the test organisms was calculated to be 0.319 mg MITC/L. The NOEC was determined to be 0.032 mg MITC/L and the LOEC was 0.112 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBH5869, chemical purity: ≥ 96.5 % (according to certificate of analysis)
Test species:	Freshwater mudsnail (*Potamopyrgus antipodarum*)
Age of organisms:	Adult females (about 3.5 – 4.5 mm length); snails of the haplotype t and morphotype “Warwick A”
Type of test:	Semi-static toxicity test (medium renewal after 48 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control 100 µL/L ethanol), 0.01, 0.03, 0.10, 0.32 and 1.00 mg MITC/L
Dilution medium:	Reconstituted water according to OECD guideline 242
Number of organisms per group:	5 organisms per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 16.3 – 16.4 °C dissolved oxygen: 6.43 - 8.70 mg/L O_2 (79 – 98 % air saturation value) pH: 7.71 – 8.07 total organic carbon: 0.567 – 0.589 mg/L photoperiod: 16 hours light and 8 hours dark light intensity: 588 – 594 lux
Test procedure:	Adult female *Potamopyrgus antipodarum* (about 3.5 – 4.5 mm length) were exposed to the test substance added to water at a range of five concentrations for 96 hours under semi-static conditions with renewal of test medium once after 48 hours. The total number of immobile individuals was assessed daily.

	The test was performed in four replicates per concentration with five specimens per replicate (according to OECD guideline 202). Reconstituted (synthetic) water was used as test medium according to OECD guideline 242. The concentrations to be tested in the definitive test were selected based on the results from the range-finding test: control, solvent control, 0.01, 0.03, 0.10, 0.32 and 1.00 mg MITC/L. The control consisted of test medium only. In addition, a solvent control containing the solubilising agent ethanol was applied at the level used in the treatments (100 µL/L).
Test item analysis:	The concentrations of MITC in the water phase were measured by chemical analysis. Samples of freshly prepared test media were taken from all five test solutions and controls at the beginning of the test and at media renewal after 48 hours prior to distribution to the test vessels. Samples of aged test media were taken directly from representative or pooled replicates per test concentration and controls at media renewal and at test end. The test item was analysed using GC-MS. If analysis could not be applied immediately after sampling, samples were stored under circumstances to maintain the test item stable.
Observations:	The number of immobile snails was visually determined daily. Any abnormalities in appearance and behaviour were recorded, if occurred. Oxygen concentration, pH value and temperature of the test solutions was checked at test start, at media renewal and at test end.
Statistical evaluation:	Where the test results show inhibition around 50 % they were statistically analysed to determine an LC_{50} value together with 95 % confidence intervals using Probit-analysis assuming log-normal distribution of the values. The NOEC and LOEC values were determined using the step-down Cochran-Armitage test procedure. The computer program ToxRat was used for statistical evaluations.

Findings:

Analytical results:	At test initiation, concentrations of 0.010, 0.029, 0.113, 0.350 and 1.06 mg MITC/L (97.7 – 113 % of nominal) were measured. At media renewal after 48 hours, measured concentrations of aged samples were at 0.010, 0.033, 0.111, 0.368 and 1.21 mg MITC/L (101 – 121 % of nominal; 97.7 – 113 % of initial concentrations). Fresh samples at 48 hours showed measured concentrations of 0.011, 0.034, 0.106, 0.372 and 1.14 mg MITC/L (106 – 116 % of nominal concentrations). At test end, concentrations of 0.011, 0.032, 0.119 and 0.372 mg MITC/L (107 – 119 % of nominal; 95.4 – 112 % of initial concentrations) were measured. The measured concentrations were mainly within a range of 80 – 120 % of nominal concentrations. However, at the highest test concentration the measured concentration deviated by more than 20 % of the nominal concentration. The test concentrations remained stable during each of the incubation periods of 48 hours. Therefore, the evaluation was based on the geometric

	mean measured concentrations of the test item, which were 0.010, 0.032, 0.112, 0.365 and 1.10 mg MITC/L (105 – 114 % of nominal concentrations).
Immobilisation, physical/pathological symptoms and changes in behaviour:	In the control, solvent control and up to and including the test concentration of 0.032 mg MITC/L (mean measured) no immobilised test organisms were observed during the test period of 96 hours. No significant signs of disease or stress like discoloration or abnormal behaviour were observed in any replicate. The test item MITC, had a statistically significant effect on the immobility of the test organisms in a concentration dependent manner. The LC_{50} for immobility was calculated to be 0.319 mg MITC/L and the NOEC was determined to be 0.032 mg MITC/L and the LOEC was 0.112 mg MITC/L. All results were calculated based on geometric mean measured concentrations.

Table B.2.9.2.2-40: Results of the chemical analysis of the test item

Nominal Concentration	Test initiation (0 hours)		Media renewal (48 hours)		
	Fresh test solutions		Aged test solutions		
	Measured concentration	% of nominal	Measured concentration	% of nominal	% of initial
[mg MITC/L]	[mg MITC/L]	[%]	[mg MITC/L]	[%]	[%]
control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.01	0.010	103.7	0.010	101.3	97.7
0.03	0.029	97.7	0.033	108.4	111.0
0.10	0.113	113.0	0.111	111.0	98.2
0.32	0.350	109.3	0.368	114.9	105.1
1.00	1.064	106.4	1.207	120.7	113.4
Nominal Concentration	**Media renewal (48 hours)**		**Test termination (96 hours)**		
	Fresh test solutions		**Aged test solutions**		
	Measured concentration	**% of nominal**	**Measured concentration**	**% of nominal**	**% of initial**
[mg MITC/L]	**[mg MITC/L]**	**[%]**	**[mg MITC/L]**	**[%]**	**[%]**
control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.01	0.011	107.3	0.011	107.0	99.7
0.03	0.034	111.8	0.032	106.7	95.4
0.10	0.106	106.3	0.119	118.7	111.6
0.32	0.372	116.4	0.372	116.1	99.8
1.00	1.137	113.7	*	*	*

LOQ: 0.005 mg MITC/L

**: all test organisms died during the first 48 hours, therefore no organisms were exposed after medium renewal and, thus, no aged samples was available at test termination.*

Table B.2.9.2.2-41: Geometric mean measured concentrations of the test item

Nominal Concentration	Geometric mean measured concentrations	
[mg MITC/L]	[mg MITC/L]	[%]
control	< LOQ	-
solvent Control	< LOQ	-

0.01	0.010	104.8
0.03	0.032	106.0
0.10	0.112	112.2
0.32	0.365	114.1
1.00	1.133	113.3

LOQ: 0.005 mg MITC/L

Table B.2.9.2.2-42: Immobilisation of the freshwater mudsnail (*Potamopyrgus antipodarum*), exposed to MITC for 96 hours under semi-static test conditions

Geometric mean measured concentration [mg MITC/L]	Total *Potamopyrgus antipodarum* introduced	Mobile	Immobile	% Immobility
solvent control	20	20	0	0.0
0.010	20	20	0	0.0 (-)
0.032	20	20	0	0.0 (-)
0.112	20	16	4	20.0 (+)
0.365	20	0	20	100 (+)
1.133	20	0	20	100 (+)

(+): significant;(-): non-significant;
Step-down Cochran-Armitage test procedure, significance level was 0.050, one-sided greater.

The response curve for immobilisation at 96 hours of *Potamopyrgus antipodarum* is shown in the following figure.

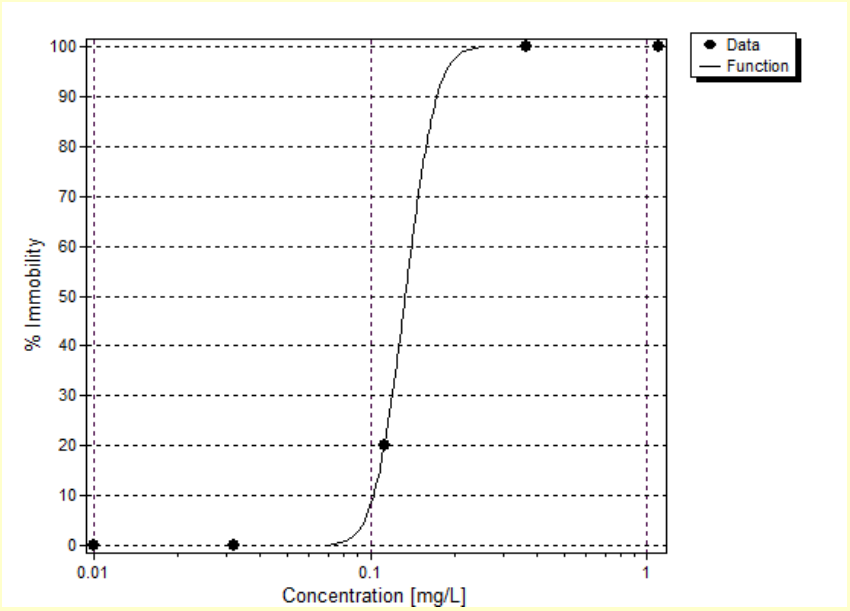


Figure B.2.9.2.2-2: Concentration-effect curve showing the influence of the test item on the immobility of the introduced test organisms as observed after 96 hours

Assessment and conclusions:

The test item Methyl Isothiocyanate had a statistically significant effect on immobility of the test organism *Potamopyrgus antipodarum* in a concentration dependent manner.
The LC_{50} for immobilisation of the test organisms was calculated to be 0.319 mg MITC/L. The NOEC was determined to be 0.032 mg MITC/L and the LOEC was 0.112 mg MITC/L. All results were calculated based on geometric mean measured concentrations.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

LC_{50} (*Potamopyrgus antipodarum*, 96 h) = 0.319 mg MITC/L (mean measured)

Analytical method:

This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.

Assessment and conclusion by Lainco:

The test item Methyl Isothiocyanate (MITC) had a statistically significant effect on immobility of the test organism *Potamopyrgus antipodarum* in a concentration dependent manner.

The LC_{50} for immobility was calculated to be 0.319 mg MITC/L.

The NOEC was determined at 0.032 mg MITC/L.

All results were calculated based on geometric mean measured MITC concentrations.

Assessment and conclusion by the RMS:

The study is considered to be sufficiently in line with the current guidance.

The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 6.43 - 8.70 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.

The validity criteria of OECD Guideline 242 were mostly met:

- the mean mortality (accounting for all control replicates) should not exceed 20 % at the end of the test (measured: 0 %)
- the mean number of embryos in the controls should be at least 5 embryos per female at the end of the test (not reported)
- the dissolved oxygen content should be at least 60 % of the air saturation value in both control and exposure groups throughout the test (measured: 6.43 - 8.70 mg/L O_2 (79 – 98 % air saturation value))
- water mean temperature should be 16 ± 1.5 °C throughout the test in both control and exposure groups (measured: 16.3 – 16.4 °C)

Despite the use of ethanol as solvent, which is not recommended by the OECD guideline 242 because it may result in the development of fungi and bacteria, it is considered that this didn't affect the validity of the test.

The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/74 (Taminco) and KCA 4.1.2/27 (Lainco), for further details).

LC_{50} (*Potamopyrgus antipodarum*, 96 h, semi-static) = 0.319 mg MITC/L (based on mean measured concentrations)

NOEC (*Potamopyrgus antipodarum*, 96 h, semi-static) = 0.032 mg MITC/L (based on mean measured concentrations)

This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/15
Report author:	[redacted]

Report year:	2019c
Report title:	*Dugesia tigrina*, acute toxicity test (96 hours) – Semi-static test conditions. Test item: Methyl Isothiocyanate (MITC)
Report No.:	TAM-003/4-22/Y
Document No.:	-
Guidelines followed in study:	OECD 202 (*Daphnia magna*, Acute Immobilisation Test)
Deviations from current test guideline:	Deviations from current OECD Guideline 202 (2004): Test species: *Dugesia tigrina* (recommendation: *Daphnia magna*)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

A study was performed to investigate the influence of the test item Methyl Isothiocyanate (MITC) on the mobility of flatworms of the species *Dugesia tigrina*. The test was conducted under semi-static conditions over a period of 96 hours with one media exchange after 48 hours.
The flatworms were placed in water containing the test item in nominal concentrations of 0.008, 0.025, 0.08, 0.25 and 0.80 mg MITC/L. A control with test medium only and a solvent control containing the solubilising agent ethanol were run in parallel. Effects on immobilisation were determined daily.
The concentrations of the test item in the media were confirmed by measurements at beginning of the test, before and after media renewal after 48 hours and at test end by GC-MS (LOQ = 0.004 mg/L). Concentrations of freshly prepared test media showed recovery rates between 102 % and 120 % of nominal concentrations. Concentrations in aged test media remained stable with 81.8 % to 105 % of initial concentrations and showed recovery rates between 96.7 % and 114 % of nominal concentrations. The measured concentrations were mainly within a range of 80 – 120 % of nominal concentrations, and remained stable during the incubation period of 48 hours. Therefore, the evaluation of effects was based on the nominal concentrations of 0.008, 0.025, 0.08, 0.25 and 0.80 mg MITC/L.
Since no statistically significant effect between the control and solvent control could be observed, treatments were compared against the solvent control.
The test item had a statistically significant effect on the immobilisation of the flatworms in a concentration dependent manner. The mortality was ≤ 20 % in the control and in the solvent control. The dissolved oxygen concentration was > 60 % of the air saturation value (ASV) in both control and exposure groups throughout the test. The mean water temperature was in the range of 20 ± 2 °C. Thus, the test was considered valid according to OECD guideline 202.
The test item MITC was acutely toxic to the test organism *Dugesia tigrina* in a 96 hour acute toxicity test. The LC_{50} for immobilisation of the test organisms was calculated to be 0.137 mg MITC/L. The NOEC was determined to be 0.025 mg MITC/L and the LOEC was 0.080 mg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBH5869, chemical purity: ≥ 96.5 % (according to certificate of analysis)
Test species:	Brown flatworm (*Dugesia tigrina*)

Age of organisms:	Flatworms used in the test were taken from a healthy stock (i.e. showing no signs of stress such as high mortality, infestation with parasites, etc.)
Type of test:	Semi-static toxicity test (medium renewal after 48 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control 100 µL/L or 0.01 % (v/v) ethanol), 0.008, 0.025, 0.08, 0.25 and 0.80 mg MITC/L
Dilution medium:	Cu-reduced dilution water
Number of organisms per group:	5 organisms per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 20.3 – 21.8 °C dissolved oxygen: 5.56 - 8.81 mg/L O_2 (65 – 107 % air saturation value) pH: 7.34 – 8.12 photoperiod: 16 hours light and 8 hours dark light intensity: 696 – 732 lux
Test procedure:	Adult flatworms *Dugesia tigrina* were exposed to the test substance added to water at a range of five concentrations for 96 hours under semi-static conditions with renewal of test medium once after 48 hours. The total number of immobile individuals was assessed daily. The test was performed in four replicates per concentration with five specimens per replicate (according to OECD guideline 202). Cu-reduced dilution water was used as test medium. The concentrations to be tested in the definitive test were selected based on the results from the range-finding test: control, solvent control, 0.008, 0.025, 0.08, 0.25 and 0.80 mg MITC/L. The control consisted of test medium only. In addition, a solvent control containing the solubilising agent ethanol was applied at the level used in the treatments (100 µL/L).
Test item analysis:	The concentrations of MITC in the water phase were measured by chemical analysis. Samples of freshly prepared test media were taken from all five test solutions and controls at the beginning of the test and at media renewal after 48 hours prior to distribution to the test vessels. Samples of aged test media were taken directly from representative or pooled replicates per test concentration and controls at media renewal and at test end. The test item was analysed using GC-MS. If analysis could not be applied immediately after sampling, samples were stored under circumstances to maintain the test item stable.
Observations:	The number of immobile flatworms was visually determined daily. Any abnormalities in appearance and behaviour were recorded, if occurred. Oxygen concentration, pH value and temperature of the test solutions was checked at test start, at media renewal and at test end.
Statistical evaluation:	Where the test results show inhibition around 50 % they were statistically analysed to determine an LC_{50} value together with 95 % confidence intervals using Probit-analysis assuming log-normal distribution of the values.

The NOEC and LOEC values were determined using the step-down Cochran-Armitage test procedure.
The computer program ToxRat was used for statistical evaluations.

Findings:

Analytical results:

At test initiation, concentrations of 0.009, 0.026, 0.093, 0.256 and 0.870 mg MITC/L (102 – 117 % of nominal) were measured. At media renewal after 48 hours, measured concentrations of aged samples were at 0.008, 0.026, 0.089, 0.251 and 0.911 mg MITC/L (99.6 – 114 % of nominal; 89.8 – 105 % of initial concentrations). Fresh samples at 48 hours showed measured concentrations of 0.009, 0.03, 0.096, 0.272 and 0.912 mg MITC/L (109 – 120 % of nominal concentrations). At test end, measured concentrations in aged media were 0.009, 0.024, 0.084 and 0.245 mg MITC/L (96.7 – 109 % of nominal; 81.8 – 92.6 % of initial concentrations).
The measured concentrations of freshly prepared test solutions were within a range of 80 – 120 % of the nominal concentrations and remained stable within ± 20 % throughout the test period of 48 hours until media renewal. Therefore, the evaluation was based on the nominal concentrations of the test item, which were 0.008, 0.03, 0.08, 0.25 and 0.80 mg MITC/L.

Immobilisation, physical/pathological symptoms and changes in behaviour:

In the control, solvent control and up to and including the test concentration of 0.008 mg MITC/L (nominal) no immobilised test organisms were observed during the test period of 96 hours. Organisms found dead in concentrations of 0.025, 0.08 and 0.25 mg MITC/L had been escaped from the water phase and were found dried out in the lid of the test vessels.
No other signs of disease or stress like discoloration or abnormal behaviour were observed in any replicate.
The test item MITC, had a statistically significant effect on the immobility of the test organisms in a concentration dependent manner.
The LC_{50} for immobility was calculated to be 0.137 mg MITC/L and the NOEC was determined at 0.025 mg MITC/L. All results were calculated based on nominal concentrations.

Table B.2.9.2.2-43: Results of the chemical analysis of the test item

Nominal Concentration	Test initiation (0 hours)		Media renewal (48 hours)		
	Fresh test solutions		Aged test solutions		
	Measured concentration	% of nominal	Measured concentration	% of nominal	% of initial
[mg MITC/L]	[mg MITC/L]	[%]	[mg MITC/L]	[%]	[%]
control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.008	0.009	111	0.008	99.6	89.8
0.025	0.026	103	0.026	104	101
0.08	0.093	117	0.089	112	95.7
0.25	0.256	102	0.251	100	97.9
0.80	0.870	109	0.911	114	105

Nominal Concentration	Media renewal (48 hours)		Test termination (96 hours)		
	Fresh test solutions		Aged test solutions		
	Measured concentration	% of nominal	Measured concentration	% of nominal	% of initial
[mg MITC/L]	[mg MITC/L]	[%]	[mg MITC/L]	[%]	[%]
Control	< LOQ	-	< LOQ	-	-
solvent control	< LOQ	-	< LOQ	-	-
0.008	0.009	118	0.009	109	92.6
0.025	0.030	118	0.024	96.7	81.8
0.08	0.096	120	0.084	105	86.9
0.25	0.272	109	0.245	97.9	90.0
0.80	0.912	114	*	*	*

LOQ: 0.004 mg MITC/L
**: all test organisms died during the first 48 hours, therefore no organisms were exposed after medium renewal and, thus, no aged samples was available at test termination.*

Table B.2.9.2.2-44: Immobilisation of the brown flatworm (*Dugesia tigrina*), exposed to MITC for 96 hours under semi-static test conditions

Nominal concentration [mg MITC/L]	Total *Dugesia tigrina* introduced	Mobile	Immobile	% Immobility
solvent control	20	20	0	0.0
0.008	20	20	0	0.0 (-)
0.025	20	19	1	5.0 (-)
0.08	20	18	2	10 (+)
0.25	20	3	17	85 (+)
0.80	20	0	20	100 (+)

(+): significant; (-): non-significant;
Step-down Cochran-Armitage test procedure, significance level was 0.050, one-sided greater.

The response curve for immobilisation at 96 hours of *Dugesia tigrina* is shown in the following figure.

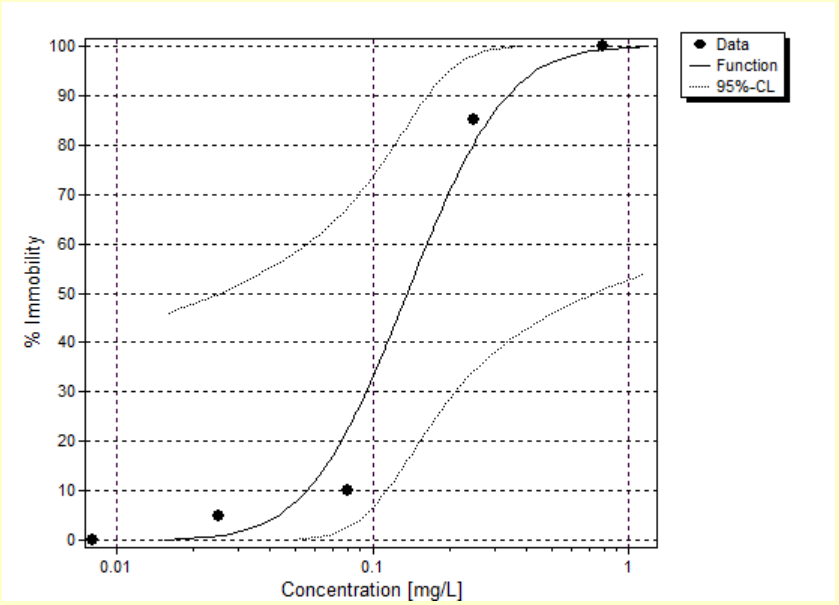


Figure B.2.9.2.2-3: Concentration-effect curve showing the influence of the test item on the immobility of the introduced test organisms as observed after 96 hours

Assessment and conclusions:

The test item Methyl Isothiocyanate had a statistically significant effect on immobility of the test organism *Dugesia tigrina* in a concentration dependent manner.
The LC_{50} for immobility of the test organisms was calculated to be 0.137 mg MITC/L. The NOEC was determined at 0.025 mg MITC/L. All results were calculated based on nominal concentrations.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Dugesia tigrina*, 96 h) = 0.137 mg MITC/L (nominal)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The test item Methyl Isothiocyanate (MITC) had a statistically significant effect on immobility of the test organism *Dugesia tigrina* in a concentration dependent manner.
The LC_{50} for immobility was calculated to be 0.137 mg MITC/L.
The NOEC was determined at 0.025 mg MITC/L.
All results were calculated based on nominal MITC concentrations.

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 5.56 - 8.81 mg/L O_2)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/73 (Taminco) and KCA 4.1.2/28 (Lainco), for further details).
LC_{50} (*Dugesia tigrina*, 96 h, semi-static) = 0.137 mg MITC/L (based on nominal concentrations)
NOEC (*Dugesia tigrina*, 96 h, semi-static) = 0.025 mg MITC/L (based on nominal concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/16
Report author:	[redacted]
Report year:	2014b
Report title:	MITC: Acute Toxicity to *Crangonyx pseudogracilis*.
Report No.:	PQB0024
Document No.:	-
Guidelines followed in study:	Procedure 202 for the "Guidelines for Testing of Chemicals" of the Organisation for Economic Co-operation and Development: *Daphnia* sp., Acute Immobilisation Test (2004);

	Procedure 235 for the "Guidelines for Testing of Chemicals" of the Organisation for Economic Co-operation and Development: *Chironomus* sp., Acute Immobilisation Test (2011)
Deviations from current test guideline:	Deviations from current OECD Guideline 202 (2004) and current OECD Guideline 235 (2011): None
Previous evaluation:	No, not previously submitted at EU level Evaluated and accepted to support Lainco S.A.'s products at Step 2 under Directive 91/414/EEC (Final RR Part B6, 2017, zRMS Spain) IIIA 10.2.2.2/02
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Lainco S.A. (letter of co-ownership by Taminco is included, study may be used by Taminco without restriction for registration purposes)

Study Summary:

The acute toxicity of MITC to the aquatic invertebrate *Crangonyx pseudogracilis* was assessed in a 48 hour laboratory test under semi-static conditions (renewal of test media after 24 hours) in accordance with OECD test guidelines 202 (2004) and 235 (2011). Groups of twenty *Crangonyx* (between 0.5 and 1 cm in length) were exposed for 48 hours to MITC, at nominal concentrations of 42.7, 93.9, 207, 455 and 1000 µg MITC/L. Overall geometric mean measured concentrations of 40.6, 99.9, 211, 566 and 1135 µg MITC/L were reported. After 48 hours, cumulative mortality was 10 %, 5 %, 5 %, 0 %, 10 %, 95 % and 100 % in the control, solvent control, 40.6, 99.9, 211, 566 and 1135 µg MITC/L (mean measured) treatment groups, respectively. The 48 hour LC_{50} value was calculated to be 312 µg MITC/L (mean measured) (95 % confidence limits: 249 - 395 µg MITC/L). The no observed effect concentration (NOEC) value was determined to be 211 µg MITC/L (mean measured).

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Freshwater crustacean amphipod (*Crangonyx pseudogracilis*)
Age of organisms:	Crustaceans used in the test were of similar size (0.5 and 1 cm in length) and from the same culture
Type of test:	Semi-static toxicity test (medium renewal after 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control 100 µL/L acetone), 42.7, 93.9, 207, 455 and 1000 µg MITC/L
Number of organisms per group:	20 organisms individually housed for the control, for the solvent control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 20.5 – 21.2 °C dissolved oxygen: 90 – 100 % air saturation value pH: 8.06 – 8.27 total hardness: 150 – 200 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 630 lux
Test procedure:	*Crangonyx pseudogracilis* (0.5 – 1 cm in length) were exposed to the test item in a semi-static test for 48 hours, with test media

	renewal after 24 hours. Twenty *Crangonyx pseudogracilis*, one animal per vessel (Wheaton vials), were exposed in each control and test group. The concentrations to be tested in the definitive test were selected based on the results from the range-finding test: control, solvent control, 42.7, 93.9, 207, 455 and 1000 µg MITC/L. The control was a blend of filtered pond water and dechlorinated tap water. An additional control group containing acetone (100 µL/L) and dilution water was included in the study.
Test item analysis:	The test concentrations of MITC were measured using GC-NPD liquid chromatography. At 0 and 24 hours during the definitive test, two samples were taken from the freshly-prepared control and test media. At 24 and 48 hours, the contents of the vessels from each group were pooled and further samples were taken for analysis. Samples were stabilised by the addition of sodium chloride and ethyl acetate. On each occasion, one of the samples was analysed and the other was stored in a freezer in case further analysis was required.
Observations:	The survival of the animals was determined in a semi-static 48 hour test by visual observation after 24 and 48 hours. The criterion of death employed in this study was absence of response to physical stimulation within 15 seconds. Dead animals were removed when observed and mortality recorded. Any abnormal behaviour or appearance was also recorded. Environmental conditions were monitored throughout the test. The temperature, pH and dissolved oxygen levels were recorded for each group in fresh media at 0 and 24 hours, and in pooled expired media at 24 and 48 hours.
Statistical evaluation:	Statistical analysis was performed using the SAFEStat LD_{50} application (version 1.5), SAS 9.1.3. The test results were expressed in terms of the mean measured concentrations. The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the animals.

Findings:

Analytical results:	In samples of freshly prepared media (0 and 24 hours), the measured concentrations of MITC ranged between 95 and 136 % of their nominal values. In samples of expired media (at 24 and 48 hours), the measured concentrations ranged between 80 and 122 % of their nominal values (between 72 and 104 % of their starting values). Based on a geometric mean, the overall measured concentrations of MITC were 40.6, 99.9, 211, 566 and 1135 µg/L, and these values have been used in the determination of the study endpoints.
Immobilisation:	Observations of the *Crangonyx pseudogracilis* in each control and test vessel were made after approximately 24 and 48 hours. After 48 hours, 95 % mortality was observed at the measured concentration of 566 µg MITC/L and the highest measured concentration at which mortality was < 15 % was 211 µg MITC/L. The results are summarised in the table below.

Table B.2.9.2.2-45: Results of the chemical analysis of the test item

Nominal (µg MITC/L)	Measured concentration (µg MITC/L)					Measured concentration as % of nominal				
	0 h fresh	24 h aged	24 h fresh	48 h aged	Mean*	0 h fresh	24 h aged	24 h fresh	48 h Aged	Mean*
control (0.0)	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-
solvent control (0.0)	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-
42.7	46.4	34.2	40.4	42.2	40.6	109	80	95	99	95
93.9	96.0	79.2	114	115	99.9	102	84	121	122	106
207	229	165	230	227	211	111	80	111	110	102
455	620	511	585	552	566	136	112	129	121	124
1000	1151	1088	1167	n.r.	1135	115	109	117	n.r.	114

h: hours; n.r.: not reported
LOQ = 1 µg MITC/L; LOD = 1 µg MITC/L.
** Geometric mean*

Table B.2.9.2.2-46: Immobilisation of the freshwater crustacean amphipod (*Crangonyx pseudogracilis*), exposed to MITC for 48 hours under semi-static test conditions

Nominal concentration (µg MITC/L)	Measured concentrations (µg MITC/L)	Cumulative No. dead after	% dead *Crangonyx pseudogracilis* after	Cumulative No. dead after	% dead *Crangonyx pseudogracilis* after
		24 hours		48 hours	
control	not detected	0	0	2	10
solvent control	not detected	0	0	1	5
42.7	40.6	0	0	1	5
93.9	99.9	0	0	0	0
207	211	0	0	2	10
455	566	6	30	19	95
1000	1135	19	100	19	100

Assessment and conclusions:

The 24 hour measured LC_{50} value for the aquatic invertebrate *Crangonyx pseudogracilis* exposed in a semi-static study was calculated to be 662 µg MITC/L (mean measured) (95 % confidence limits of 562 and 721 µg/L). The 24 hour NOEC value was determined to be 211 µg MITC/L (mean measured).
The 48 hour measured LC_{50} value for the aquatic invertebrate *Crangonyx pseudogracilis* exposed in a semi-static study was calculated to be 312 µg MITC/L (mean measured) (95 % confidence limits of 249 and 395 µg/L). The 48 hour NOEC value was determined to be 211 µg MITC/L (mean measured).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.

Endpoints:
LC_{50} (*Crangonyx pseudogracilis*, 48 h) = 0.312 mg MITC/L (mean measured) (95 % confidence limits: 0.249 – 0.395 mg MITC/L)
Analytical method:
The method is acceptable for the quantification of MITC in aquatic arthropod medium.
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
LC_{50} (*Crangonyx pseudogracilis*, 48 h, semi-static) = 0.312 mg MITC/L (mean measured) (95 % confidence limits: 0.249 – 0.395 mg MITC/L)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:

- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 10 %)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 90 – 100 % air saturation value)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/82 (Taminco) and KCA 4.1.2/21 (Lainco), for further details).
LC_{50} (*Crangonyx pseudogracilis*, 48 h, semi-static) = 0.312 mg MITC/L (based on mean measured concentrations)
NOEC (*Crangonyx pseudogracilis*, 48 h, semi-static) = 0.211 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.4.2/17
Report author:	[redacted]
Report year:	2014c
Report title:	MITC: Acute Toxicity to *Lumbriculus variegatus*.
Report No.:	PQB0025
Document No.:	-
Guidelines followed in study:	"Guidelines for Testing of Chemicals" of the Organisation for Economic Co-operation and Development: Procedure 202 *Daphnia* sp., Acute Immobilisation Test (2004) and Procedure 235 *Chironomus* sp., Acute Immobilisation Test (2011)
Deviations from current test guideline:	Deviations from current OECD Guideline 202 (2004) and current OECD Guideline 235 (2011): None
Previous evaluation:	No, not previously submitted at EU level Evaluated and accepted to support Lainco S.A.'s products at Step 2 under Directive 91/414/EEC (Final RR Part B6, 2017, zRMS Spain) IIIA 10.2.2.2/04
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities

Acceptability/Reliability:	Study is acceptable
Study owner:	Lainco S.A. (letter of co-ownership by Taminco is included, study may be used by Taminco without restriction for registration purposes)

Study Summary:

The acute toxicity of MITC to *Lumbriculus variegatus* was assessed in a 48 hour laboratory test under semi-static conditions (renewal of test media after 24 hours) in accordance with OECD test guidelines 202 (2004) and 235 (2011). Due to the volatile nature of the test substance the study was conducted in completely filled and sealed vessels. Groups of twenty *Lumbriculus variegatus* (between 10 and 15 mm in length) were exposed for 48 hours to MITC, at nominal concentrations of 0.0470, 0.0940, 0.188, 0.375, 0.750 and 1.50 mg MITC/L. Overall mean measured concentrations were 0.0422, 0.0970, 0.170, 0.360, 0.725 and 1.46 mg MITC/L. After 48 hours, cumulative mortality was 0 %, 5 %, 0 %, 0 %, 30 %, 100 %, 100 % and 100 % in the control, solvent control, 0.0422, 0.0970, 0.170, 0.360, 0.725 and 1.46 mg MITC/L (mean measured) treatment groups, respectively. The 48 hour LC_{50} value was calculated to be 0.205 mg MITC/L (mean measured) (95 % confidence limits: 0.169 - 0.218 mg MITC/L. The no observed effect concentration (NOEC) value was determined to be 0.0970 mg MITC/L (mean measured).

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Freshwater oligochaete (*Lumbriculus variegatus*)
Age of organisms:	Worms were synchronised approximately 2 weeks before the range finding and definitive tests, to provide animals between 10 and 15 mm in length
Type of test:	Semi-static toxicity test (medium renewal after 24 hours)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control 100 µL/L acetone), 0.0470, 0.0940, 0.188, 0.375, 0.750 and 1.50 mg MITC/L
Number of organisms per group:	5 organisms per replicate, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	48 hours
Test conditions:	temperature: 20.8 – 21.1 °C dissolved oxygen: 80 – 98 % air saturation value pH: 7.92 – 8.20 total hardness: 158 mg/L $CaCO_3$ alkalinity: 133 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 630 lux
Test procedure:	*Lumbriculus variegatus* were exposed to the test substance added to water at a range of six concentrations for 48 hours under semi-static conditions with renewal of test medium once after 24 hours. The total number of immobile individuals was assessed daily. The test was performed in four replicates per concentration with five specimens per replicate (according to OECD guideline 202). The concentrations to be tested in the definitive test were selected based on the results from the range-finding test: control, solvent control, 0.0470, 0.0940, 0.188, 0.375, 0.750 and 1.50 mg MITC/L. The control consisted of test medium only. In addition, a solvent control

	containing the solubilising agent acetone was applied at the level used in the treatments (100 µL/L).
Test item analysis:	The test concentrations of MITC were measured using GC-NPD liquid chromatography. At 0 and 24 hours during the definitive test, two samples (20 mL) were taken from the freshly-prepared control and test media. At 24 and 48 hours, the contents of the test vessels from each group were pooled and further samples were taken for analysis. Samples were stabilised by the addition of sodium chloride and ethyl acetate. On each occasion, one of the samples was analysed and the other was stored in a freezer in case further analysis was required.
Observations:	The survival of the animals was determined in a semi-static 48 hour test by visual observation after 24 and 48 hours. The criterion of death employed in this study was absence of response to physical stimulation within 15 seconds. The numbers of dead and living worms were recorded together with any abnormal behaviour or appearance compared with control worms. Environmental conditions were monitored throughout the test. The temperature, pH and dissolved oxygen levels were recorded for each group in fresh media at 0 and 24 hours, and in pooled expired media at 24 and 48 hours.
Statistical evaluation:	Statistical analysis was performed using the SAFEStat LD_{50} application (version 1.5), SAS 9.1.3. The test results were expressed in terms of the mean measured concentrations. The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the mortality of the animals. An incidence rate of more than 15 % was considered to be significant.

Findings:

Analytical results:	With the exception of the expired medium at a nominal 0.0470 mg MITC/L at 48 hours (67 % of its nominal value), the intended exposure concentrations of MITC were adequately achieved (between 85 and 115 % of their nominal values) and maintained during the test (between 79 and 95 % of their starting values). Using a geometric mean, the overall measured concentrations were 0.0422, 0.0970, 0.170, 0.360, 0.725 and 1.46 mg MITC/L, and these values have been used in the determination of study endpoints.
Immobilisation:	Observations of the *Lumbriculus variegatus* in each control and test vessel were made after 24 and 48 hours. After 48 hours, the lowest measured concentration at which 100 % mortality was observed was 0.360 mg MITC/L. The highest measured concentration at which no significant mortality (≤ 15 %) was observed was 0.0970 mg MITC/L. The results are summarised in the table below.

Table B.2.9.2.2-47: Results of the chemical analysis of the test item

Nominal (mg MITC/L)	Measured concentration (mg MITC/L)					Measured concentration as % of nominal				
	0 h	24 h	24 h	48 h	Mean*	0 h	24 h	24 h	48 h	Mean*

	fresh	aged	fresh	aged		fresh	Aged	fresh	Aged	
control (0.0)	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-
solvent control (0.0)	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-
0.0470	0.0505	0.0398	0.0504	0.0314	0.0422	107	85	107	67	90
0.0940	0.1010	0.0828	0.108	0.0982	0.0970	107	88	115	104	103
0.188	0.183	0.161	0.173	0.165	0.170	97	86	92	88	90
0.375	0.380	0.341	NM	NM	0.360	101	91	-	-	96
0.750	0.760	0.691	NM	NM	0.725	101	92	-	-	97
1.50	1.57	1.35	NM	NM	1.46	105	90	-	-	97

h: hours
NM: not measured because all of the worms at this level had died before renewal of the test media at 24 hours
LOQ = 0.01 mg MITC/L; LOD = 0.005 mg MITC/L
** Geometric mean*

Table B.2.9.2.2-48: Immobilisation of the freshwater oligochaete (*Lumbriculus variegatus*), exposed to MITC for 48 hours under semi-static test conditions

Nominal concentration (mg MITC/L)	Measured concentrations (mg MITC/L)	Cumulative no. dead *Lumbriculus variegatus* after		Cumulative % dead *Lumbriculus variegatus* after	
		24 hours	48 hours	24 hours	48 hours
control	not detected	0	0	0	0
solvent control	not detected	1	1	5	5
0.0470	0.0422	0	0	0	0
0.0940	0.0970	0	0	0	0
0.188	0.170	2	6	10	30
0.375	0.360	20	20	100	100
0.750	0.725	20	20	100	100
1.50	1.46	20	20	100	100

Assessment and conclusions:

The 24 hour measured LC_{50} value for *Lumbriculus variegatus* in a semi-static study was calculated to be 0.232 mg MITC/L (mean measured) (95 % confidence limits of 0.170 and 0.254 mg/L). The 24 hour NOEC value was determined to be 0.170 mg MITC/L (mean measured).
The 48 hour measured LC_{50} value for *Lumbriculus variegatus* in a semi-static study was calculated to be 0.205 mg MITC/L (mean measured) (95 % confidence limits of 0.169 and 0.218 mg/L). The 48 hour NOEC value was determined to be 0.0970 mg MITC/L (mean measured).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
LC_{50} (*Lumbriculus variegatus*, 48 h) = 0.205 mg MITC/L (mean measured) (95 % confidence limits: 0.169 – 0.218 mg MITC/L)
Analytical method:
The method is acceptable for the quantification of MITC in aquatic arthropod medium.
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
LC_{50} (*Lumbriculus variegatus*, 48 h, semi-static) = 0.205 mg MITC/L (mean measured) (95 % confidence limits: 0.169 – 0.218 mg MITC/L)

Assessment and conclusion by the RMS:
The study is considered to be sufficiently in line with the current guidance.
The validity criteria of OECD Guideline 202 were met:
- the immobility in the controls should not exceed 10 % at the end of the exposure (measured: 0 % in control and 5 % in solvent control)
- the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (measured: 80 – 98 % air saturation value)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 202 when reporting the data.
The validity criteria of OECD Guideline 225 were not all met:
- the average number of living worms per replicate in the controls should have increased by a factor of at least 1.8 at the end of the exposure compared to the number of worms per replicate at the start of exposure (not reported in the study)
- the pH of the overlying water should be between 6 and 9 throughout the test (measured: 7.92 - 8.20)
- the oxygen concentration in the overlying water should not be below 30 % of air saturation value (ASV) at test temperature during the test (measured: 80 - 98 % air saturation value)

Since the majority of the validity criteria were met, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/83 (Taminco) and KCA 4.1.2/22 (Lainco), for further details).
LC_{50} (*Lumbriculus variegatus*, 48 h, semi-static) = 0.205 mg MITC/L (based on mean measured concentrations)
NOEC (*Lumbriculus variegatus*, 48 h, semi-static) = 0.0970 mg MITC/L (based on mean measured concentrations)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. 2 acute (short-term) toxicity studies with algae are available for metam (performed with either metam-sodium or metam-potassium), and 8 such studies with algae and 2 studies with aquatic plants are available for MITC.

Studies with metam

Data point:	KCA 8.2.6.1/01
Report author:	[redacted]
Report year:	2003
Report title:	Metam Sodium 510 g/L algal growth inhibition assay.
Report No.:	UCB 833/023032
Document No.:	-
Guidelines followed in study:	OECD 201 EU Directive 92/69/EEC, Part C2 JMAFF Environmental Test Guidelines US EPA OPPTS850.5400
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)

GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: UCB and Buckman Laboratories)

Study Summary:

In a static acute toxicity test, the green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), was exposed to a series of six concentrations of Metam Sodium 510 g/L ranging from 0.106 to 3.38 mg a.s./L. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 1 x 10^4 cells/mL. At the start of the test, the measured levels of metam-sodium in samples of the test cultures ranged between 53 % and 88 % of their nominal values: 0.0601, 0.152, 0.322, 0.689, 1.42 and 2.96 mg a.s./L. After 24 hours, no metam-sodium was detected in samples of the test cultures except at the highest test level where the measured level was 4 % of its nominal value; at 96 hours, no metam-sodium was found at the highest test level. These results were not unexpected because metam-sodium was known to be unstable in water at the levels employed in the test.

After 96 hours of exposure to Metam Sodium 510 g/L, the E_bC_{50} and E_rC_{50} were 1.67 and 3.22 mg/L (nominal levels), respectively; equivalent to 0.548 and 1.13 mg a.s./L (initial measured levels). The no observed effect concentration (NOEC) of Metam Sodium 510 g/L was nominally 1 mg/L; 0.322 mg a.s./L (initial measured level). At 4 and 8 mg/L, metam-sodium was found to be algistatic (suppressed growth).

Materials and methods:

Test substance:	Metam Sodium 510 g/L, formulation containing 510 g metam-sodium/L, batch n°: 32 E 28/6
Test species:	Green algae (*Pseudokirchneriella subcapitata*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.25, 0.5, 1, 2, 4 and 8 mg Metam Sodium formulation/L
Dilution medium:	OECD Algal Nutrient Medium
Initial cell density, number of replicates:	1 x 10^4 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	96 hours
Test conditions:	temperature: 22.4 – 24.0 °C pH: 7.66 – 8.13 at start, 7.85 – 9.91 at end photoperiod: continuous illumination light intensity: 4070 – 4270 lux
Test procedure:	The green alga *Pseudokirchneriella subcapitata* was exposed for 96 hours to nominal concentrations of 0, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L as Metam Sodium 510 g/L, corresponding to 0.106, 0.211, 0.422, 0.844, 1.69 and 3.38 mg a.s./L. Three replicate test chambers were maintained in each treatment group and six replicates were maintained in the control group. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 1 x 10^4 cells/mL.

Test item analysis:

The test concentrations of Metam Sodium 510 g/L were verified by measuring the concentrations of metam-sodium using an HPLC method of chemical analysis and an analytical standard. At the start of the definitive test, one sample (*ca*. 25 mL) was taken from an additional flask containing the freshly-prepared control and test media; after 24 hours, a further sample was taken from an additional flask prepared for the control and at each concentration that had been incubated with the test vessels. At 96 hours, a sample was taken at the highest test concentration (8 mg/L), the only level where metam-sodium was detected at 24 hours. An additional sample was also taken from a flask containing Metam Sodium 510 g/L at 8 mg/L but with no algal cells, in order to obtain information on the extent of adsorption/absorption of the test substance by the algal cells.

On each occasion, the samples were analysed immediately. At 10 mg/L and lower concentrations, samples were analysis in duplicate; at 100 and 1000 mg/L, one analysis was performed for each sample.

Observations:

Samples were taken from six control flasks and three test flasks at each concentration at 24, 48, 72 and 96 hours during the definitive test and the cell densities measured using a haemacytometer (Improved Neubauer). At the start of the test, the cell density for each exposure group was verified using the additional flasks prepared for water quality and chemical analysis measurements. The estimate of cell numbers in each sample was based on counting either four or eight cells (depending on the cell density) of the haemacytometer grid; the mean of these counts was used as the estimate of the cell count. The presence of any abnormal cells was also noted during counting.

At the end of the test, aliquots were taken from each test flask at the two highest exposure concentrations (nominally 4 and 8 mg/L) where growth had been severely inhibited, and from each control flask. The samples from each of the groups were pooled (10 mL total) and an aliquot (0.5 mL) of the combined suspensions was used to inoculate fresh sterile culture medium (100 mL); two vessels (250 mL glass conical flasks) were established for each group. The flasks were plugged, incubated for up to six days under the same environmental conditions as employed during the definitive test and then the cell density in each control culture and test culture was determined after four and six days respectively.

Statistical evaluation:

The area under each growth curve (cell density x time) was taken to be an index of growth and was calculated. Percentage inhibition of growth at each test concentration was calculated by comparing the area under the test curve with that under the control curve as appropriate. The E_bC_{50} was calculated by a computer program (Stephan; 1977, 1982) using percentage effect and the nominal and initial measured metam-sodium test concentrations.

The average specific growth rate for each exponentially growing culture was also calculated from the appropriate section of the growth curve. The E_rC_{50} was calculated by a computer using percentage effect and the nominal and initial measured metam-sodium test concentrations. The "no observed effect concentration"

(NOEC) was determined using Dunnett's multi-comparison test to compare the percentage inhibition in the test group with that for the control cultures.

Findings:

Analytical results:

At the start of the test, the measured levels of metam-sodium in samples of the test cultures ranged between 53 and 88 % of their nominal values: 0.0601, 0.152, 0.322, 0.689, 1.42 and 2.96 mg a.s./L. After 24 hours, no metam-sodium was detected in samples of the test cultures except at the highest test level where the measured level was 4 % of its nominal value; at 96 hours, no metam-sodium was found at the highest test level. These results were not unexpected because metam-sodium was known to be unstable in water at the levels employed in the test.

Inhibition of biomass and growth:

Based on Dunnetts multicomparison test, the no observed effect concentration for the area under the growth curve ($NOEC_b$) and the average specific growth rate ($NOEC_r$) was 1 mg/L expressed in terms of Metam Sodium 510 g/L (nominal), 0.422 mg/L as metam sodium (nominal) and 0.322 mg a.s./L based on the initial measured levels. No microscopic abnormalities of the cells were detected.

Mean cell densities for each culture and percentage inhibition by comparing the test group value with that of the control curve average are given in the table below.

Subcultures (established in freshly-prepared culture medium) from test cultures containing Metam Sodium 510 g/L at 4 and 8 mg/L (nominal) had re-established growth after six days of incubation, indicating that at these levels, the test substance was algistatic (suppressing growth).

Table B.2.9.2.2-49: Mean measured concentrations of metam-sodium in algal media at test initiation (0 hours), 24 hours and at test termination (96 hours) of the Algal Growth Inhibition Test with *Pseudokirchneriella subcapitata*

Nominal concentrations (mg/L)		Mean measured metam-sodium concentrations (mg a.s./L)*					
Metam sodium 510 g/L	**Metam-sodium (a.s.)**	**0 hours**	**% of nominal**	**24 hours**	**96 hours**	**% of initially measured (96 hours)**	**Mean**
control	control	n.d.	-	n.d.[5]	n.d.	0	-
0.25[1]	0.106	0.060	56.7	n.d.	n.s.	0	0.027
0.5[1]	0.211	0.152	71.8	n.d.	n.s.	0	0.057
1.0[1]	0.422	0.322	76.3	n.d.	n.s.	0	0.114
2.0[2]	0.844	0.689	81.6	n.d.	n.s.	0	0.236
4.0[2]	1.69	1.415	83.7	n.d.	n.s.	0	0.478
8.0[2]	3.38	2.960	87.6	0.143	n.d.	0	1.485
8.0[2,3]	3.38	[4]	[4]		n.s.	n.d.	-

n.d.: none detected (< 0.02 mg metam-sodium/L)
n.s.: not sampled
** Mean of two samples taken*

1 Prepared from the 10 mg/L aqueous stock.
2 Prepared from the 100 mg/L aqueous stock.
3 Test media without algae incubated under the test conditions.
4 Samples taken from the test medium at 8 mg/L at the start of the test are applicable to this exposure group so no analysis was undertaken.
5 For mean calculation, half of the limit of detection (0.01 mg/L) was used when substance could not be detected.

Table B.2.9.2.2-50: Mean cell densities and percent inhibition on growth rate of *Pseudokirchneriella subcapitata*, exposed to Metam Sodium 510 g/L for 96 hours under static test conditions

Nominal concentration (mg/L)	Initially measured concentration (mg a.s./L)	0 h[1,2]	24 h[1,2]	48 h[1,2]	72 h[1,2]		96 h[1,2]	
		Cell density (cells/ mL)	Cell density (cells/ mL)	Cell density (cells/ mL)	Cell density (cells/ mL)	% Inhibition	Cell density (cells/ mL)	% Inhibition
negative control	--	0.938	3.99	19.0	64.9	--	170	--
0.25	0.0601	1.25	3.75	21.0	64.4	0	189	0
0.5	0.152	1.13	3.42	18.9	65.7	0	193	0
1.0	0.322	0.938	3.83	10.1	42.3	10	133	5
2.0	0.689	1.13	3.13	7.99	17.8	31	64.6	19
4.0	1.42	1.19	2.38	3.65	6.54	61	8.34	64
8.0	2.96	0.938	1.25	1.81	1.77	87	1.42	94

[1] *Cell densities are presented as x 10^4 cells/ml.*

[2] *Cell densities are calculated using the means of the two counts performed for each flask.*

Assessment and conclusions:

The green alga, *Pseudokirchneriella subcapitata*, was exposed to a series of six concentrations of Metam Sodium 510 g/L ranging from 0.106 to 3.38 mg a.s./L. After 96 hours of exposure to Metam Sodium 510 g/L, the E_bC_{50} and ErC_{50} were 1.67 and 3.22 mg/L (nominal levels), respectively; equivalent to 0.548 and 1.13 mg a.s/L (initial measured levels). The no observed effect concentration (NOEC) of Metam Sodium 510 g/L was nominally 1 mg/L; 0.322 mg a.s./L (initial measured level). At 4 and 8 mg/L, equivalent to 1.42 and 2.96 mg a.s./L (initial measured level), metam-sodium was found to be algistatic (suppressed growth).

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 1.69 mg Metam Sodium 510 g/L/L (initial measured)

E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.556 mg a.s./L (initial measured)

E_bC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 1.67 mg Metam Sodium 510 g/L/L (initial measured)

E_bC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 0.548 mg a.s./L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 3.08 mg Metam Sodium 510 g/L/L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 1.08 mg a.s./L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 3.22 mg Metam Sodium 510 g/L/L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 1.13 mg a.s./L (initial measured)

Analytical method:

This study is submitted for information only and is not relied upon for risk assessment; therefore an analytical review is not presented.

Assessment and conclusion by Lainco:

The study is mentioned in the dossier of the applicant Lainco.

EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :

E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h, static) = 1.08 mg metam-sodium/L (initial measured)

Lainco S.A. notes that analytical measurements confirmed that test concentrations were not maintained throughout the static test (no test substance detected after 24 hours) and hence the study is reported here for supporting information only, on request from the RMS during the completeness check.

Assessment and conclusion by the RMS:

The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: > 16)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 7 %

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore it is uncertain to consider it "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/62, for further details). No further data required since endpoint is not relied upon for risk assessment.
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 1.69 mg Metam Sodium 510 g/L/L (initial measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.556 mg a.s./L (initial measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 1.67 mg Metam Sodium 510 g/L/L (initial measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.548 mg a.s./L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 3.08 mg Metam Sodium 510 g/L/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 1.08 mg a.s./L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 3.22 mg Metam Sodium 510 g/L/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 1.13 mg a.s./L (initial measured)
NOEC (*Pseudokircheneriella subcapitata*, 96 h, static) = 1 mg Metam Sodium 510 g/L/L (initial measured)
NOEC (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.322 mg Metam Sodium 510 g/L/L (initial measured)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.6.1/02
Report author:	[redacted]
Report year:	2020a
Report title:	Metam Sodium 510 g/L algal growth inhibition assay - Statistical Re-analysis.
Report No.:	CEA.2141
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.

Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Due to the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report from Huntingdon Life Sciences Ltd., Project number: UCB 833/023032 ([redacted], 2003; KCA 8.2.6.1/01), for the toxicity of Metam Sodium 510 g/L on the growth of the unicellular green algae *Selenastrum capricornutum* did not provide estimates of the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal concentrations of Metam Sodium 510 g/L at 0.25, 0.5, 1, 2, 4 and 8 mg/L; equivalent to 0.106, 0.211, 0.422, 0.844, 1.69 and 3.38 mg a.s./L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 53 % to 88 % of the nominal values at the start of the test. After 24 hours, no Metam Sodium could be detected at any concentration except at the highest concentration, where the measured concentration was 4 % of the nominal value. In line with the original study, the initial mean measured test concentrations were used for this re-analysis; the concentrations were 0.0601, 0.152, 0.322, 0.689, 1.42 and 2.96 mg a.s./L.

The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (μ)
- Biomass (b)

Statistical analyses of the available data for both **yield** (Y) and **growth rate** (μ) revealed that no reliable EC_{10} or EC_{20} values could be calculated.

Statistical analyses of the available data for **biomass** (b) revealed that the following EC_{20} values could be reliably determined:

Table B.2.9.2.2-51: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to metam-sodium for 7 days under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours	48 hours	72 hours	96 hours
	E_bC_{20}	E_bC_{20}	E_bC_{20}	E_bC_{20}
Value [mg a.i./L]	0.084	0.078	0.075	0.080
lower 95 %-cl	0.048	0.062	0.060	0.068
upper 95 %-cl	0.123	0.094	0.089	0.093

cl: confidence limit

The EC_{10} values determined for biomass were not considered to be reliable as they were more than 25 % below the lowest test concentration.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints from re-analysis:
Statistical analyses of the available data for biomass revealed that the following EC_{20} values could be reliably determined:
E_bC_{20} (*Pseudokircheneriella subcapitata*, 24 h) = 0.084 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 48 h) = 0.078 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.075 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 96 h) = 0.080 mg a.s./L (initial mean measured)
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco, however no statistical re-analysis is presented.

Assessment and conclusion by the RMS:
The study is acceptable.
Endpoints from re-analysis:
Statistical analyses of the available data for biomass revealed that the following EC_{20} values could be reliably determined:
E_bC_{20} (*Pseudokircheneriella subcapitata*, 24 h, static) = 0.084 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 48 h, static) = 0.078 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.075 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.080 mg a.s./L (initial mean measured)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Data point:	KCA 8.2.6.1/03
Report author:	[redacted]
Report year:	2011
Report title:	Metam sodium (technical grade): Algal growth inhibition assay.
Report No.:	LNO0008
Document No.:	-
Guidelines followed in study:	EC Methods for Determination of Ecotoxicity, Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 3 "Algal Inhibition Test" OECD Guideline for Testing of Chemicals No. 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (2006)
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): The temperature of the control and test media measured at the start of the definitive test ranged between 20.2 °C and 20.5 °C which was just below the range stated in the study protocol (21 °C to 24 °C). The variation in light intensities across the test area during the definitive test exceeded the range (± 15 %) stated in the protocol, with a maximum deviation of 22.9 %. These deviations had no impact on the validity or integrity of the test because the validity criteria for this study type were met.
Previous evaluation:	No, not previously submitted at EU level

	Accepted to support Lainco S.A.'s Step 1 application (2015, RMS Belgium)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Due to some deviations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Lainco S.A. (letter of co-ownership by Taminco is included, study may be used by Taminco without restriction for registration purposes)

Study Summary:

The effects of metam-sodium (technical grade) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* were assessed under static exposure conditions over a period of 72 hours in accordance with OECD test guideline 201 (2006).
Following a range-finding test, in the definitive test algae were exposed to nominal concentrations of 0.155, 0.342, 0.751, 1.65, 3.64 and 8.0 mg test item/L; equivalent to 0.0693, 0.153, 0.336, 0.738, 1.63 and 3.58 mg a.s./L (accounting for 44.7 % purity of test item). The overall geometric mean measured concentrations of total metam-sodium (metam-sodium and its degradation product methyl isothiocyanate (MITC)) were 0.0378, 0.0813, 0.145, 0.396, 0.803 and 2.10 mg/L (between 43 % and 59 % of nominal values). The biological results are reported based on mean measured concentrations of total metam-sodium.
The study was considered valid as all validity criteria were met. The 72 hour E_rC_{50} value based on growth rate was calculated to be 0.339 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.295 - 0.388 mg/L). The 72 hour E_rC_{10} value was calculated to be 0.0779 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0568 - 0.105 mg/L). The no observed effect concentration (NOEC) value based on growth rate was determined to be 0.0813 mg/L (mean measured total metam-sodium). The 72 hour E_yC_{50} value based on yield was calculated to be 0.118 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0983 - 0.142 mg/L). The no observed effect concentration (NOEC) value based on yield was determined to be 0.0378 mg/L (mean measured total metam-sodium).

Materials and methods:

Test substance:	Metam-sodium (technical grade), batch no.: B3698, chemical purity: 44.7 % w/w metam-sodium
Test species:	Green algae (*Pseudokirchneriella subcapitata*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.155, 0.342, 0.751, 1.65, 3.64 and 8.0 mg metam-sodium (technical grade)/L; equivalent to 0 (control), 0.0693, 0.153, 0.336, 0.738, 1.63 and 3.58 mg a.s./L
Dilution medium:	OECD Algal Nutrient Medium
Initial cell density, number of replicates:	1 x 10^4 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	72 hours
Test conditions:	temperature: 20.2 – 22.5 °C pH: 7.57 – 9.06 photoperiod: continuous illumination

	light intensity: 4330 – 6400 lux
Test procedure:	An algal inoculum was cultured as follows. Sterile algal nutrient medium was inoculated with cells aseptically removed from the slope culture; these primary liquid cultures (100 mL) were incubated for approximately three days in an orbital incubator under continuous illumination at nominal temperatures in the range of 21 - 25 °C. Subsequently, appropriate volumes of these primary cultures were aseptically transferred to fresh sterile algal nutrient medium to prepare secondary liquid cultures; these cultures were incubated, as stated above, for a further three days to provide an inoculum in the log phase of growth, characterised by a cell density of 1.21 x 10^6 cells/mL. An aliquot (41.3 mL) of the secondary algal inoculum was added to 5 L of culture medium and a 99 mL aliquot of this inoculated culture medium was added to each control and test vessel. The culture medium (OECD medium) used to prepare the aqueous stock solutions was de-oxygenated using helium (3 L of medium was de-oxygenated by bubbling helium through it for 46 minutes before use). The test substance (80 mg) was dissolved in de-oxygenated OECD medium (100 mL) in a volumetric flask to provide a concentrated, aqueous stock solution at a nominal concentration of 1 g/L. The contents of the flask were vigorously shaken before being used directly at the highest test concentration or serially diluted, with de-oxygenated OECD medium, to provide the test media at the five lower concentrations. The aqueous test media were prepared in subdued light and the vessels were kept in black plastic bags to exclude light. 1 mL of the appropriate stock solution was added to the inoculated culture medium (99 mL) in each test vessel to give an initial algal cell density of 1 x 10^4 cells/mL. Each test vessel was loosely plugged with a foam bung. Six test concentrations were tested: nominally 0.155, 0.342, 0.751, 1.65, 3.64 and 8.0 mg test item/L; equivalent to 0.0693, 0.153, 0.336, 0.738, 1.63 and 3.58 mg a.s./L (accounting for 44.7 % purity of test item). In addition, two control groups were tested: an OECD culture medium control group and a de-oxygenated control group. Seven flasks were established for the control groups and five flasks for each test group, plus an additional flask at nominal concentrations of 0.155 and 8 mg test item/L, which contained test medium but no algal cells. Six of the control flasks and three from each test concentration were identified for cell density determinations; the other flasks were used for verifying the initial algal cell density and chemical analysis at 0 and 24 hours. The media remaining in the preparation flasks were used for water quality measurements at the test start.
Test item analysis:	Metam-sodium is known to be unstable in water and forms a volatile, unstable breakdown product (methyl isothiocyanate; MITC). The test media were prepared from a series of aqueous stock solutions that had been made in OECD medium that had been de-oxygenated in an attempt to reduce the rate at which metam-sodium degrades. The exposure concentrations were monitored by

	measuring the concentrations of the active substance metam-sodium using an HPLC-UV method of analysis and the concentrations of the breakdown product MITC using a GC-NPD method of analysis. At the start of the definitive test, four samples were taken from additional flasks containing the freshly-prepared control and test media. At 24 hours, four samples were taken from an additional flask prepared for each control and test group that had been incubated with the test vessels. At 72 hours, the contents of the replicate flasks for each group were pooled and a further four samples taken for analysis. Additional samples were also taken from flasks containing 0.155 and 8 mg test item/L (nominal) but with no algal cells, in order to obtain information on the extent of adsorption/absorption of the test substance by the algal cells. Two of the four samples were reserved for the analysis of metam-sodium; these samples (5 mL) were placed into vials containing 0.1 % cobalt (II) nitrate in methanol; the other two samples (20 mL) were retained for the analysis of MITC. On each occasion, one of the samples was analysed and the other was stored in a refrigerator in case further analysis was required. Test results were expressed in terms of total metam-sodium calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium.
Observations:	Samples were taken from control and test flasks at 24, 48 and 72 hours and the cell densities measured using a Coulter Z Series Particle Count and Size Analyser. The estimate of cell numbers in each sample was based on the mean of three consecutive counts, corrected for background counts of uninoculated dilution media. The presence of any abnormal cells was also noted during screening of each test level.
Statistical evaluation:	Statistical analysis was performed using SAS 9.1 (SAS Institute, 2002). Test results were expressed in terms of mean measured total metam-sodium concentration (calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium). The area under the curve was divided by initial count and total time to give AUCP (Area Under the Curve expressed as a proportion of the initial cell count), where a value of 1 represents no growth and a value of 0 represents complete toxicity (all algae killed). In order to estimate the concentration at which 50 % inhibition of growth occurred (EC_{50}), sigmoidal curves were fitted to AUCP and growth rate. For both variables, 0 % inhibition was defined as the control (de-oxygenated) mean and 100 % inhibition was defined as no growth. The minimum of the curve (for infinite concentration) was bounded between 0 and 1 for AUCP and between -1000 and 0 for growth rate. Yield was calculated for each test vessel as the final cell density (after 72 hours) minus the presumed initial cell density of 1×10^4 cells/mL. A mean yield value for each test concentration was calculated and the percentage inhibition was determined.

All 95 % confidence intervals for EC_{50} were calculated using the likelihood ratio method (Donaldson and Schnabel, 1985). The EC_{10} (with 95 % confidence interval) was also estimated by re-parameterising the formulae for AUCP and growth rate. For AUCP, % inhibition and growth rate, Williams' test (1971, 1972) was also used to compare each treated group with the de-oxygenated control unless there was evidence of a non-monotonic dose-response relationship, in which case Dunnett's test (1955, 1964) was used.

Findings:

Analytical results:

At the start of the test, the measured levels of metam-sodium in samples of the test cultures ranged between 106 and 123 % of their nominal values, with metam-sodium present as the primary constituent. After 24 hours, the measured levels had decreased, ranging between 46 and 65 % of their nominal values, with MITC as the main constituent except at the highest test level where metam-sodium was dominant. At 72 hours, the measured levels ranged between 17 and 29 % of their nominal and comprised MITC with no measurable levels of metam-sodium present at any test level. These results were not unexpected as both metam-sodium and MITC were known to be unstable in water at the levels employed in the test.

Low but measurable levels of MITC (0.007 mg/L) were found in samples taken from the control vessels at 72 hours. Since no significant effects were noted in the lowest test group where the measured MITC concentration (0.017 mg/L) was higher than that seen in the controls, the validity of the test was not considered to have been affected.

The overall geometric mean measured levels of metam-sodium (technical grade) were 0.0378, 0.0813, 0.145, 0.396, 0.803 and 2.10 mg/L.

After 72 hours, analysis of a sample of media at 0.155 and 8 mg test item/L (nominal) incubated without algal cells gave similar results to test medium incubated in the presence of algal cells, showing a decrease in the measured levels of metam-sodium and MITC during the test. These results indicate that the concentrations of the test substance cannot be maintained under the conditions of this test, irrespective of whether or not algal cells are present.

A summary of the measured concentrations in the test media is presented in the table below, expressed in terms of total metam-sodium (calculated from the measured concentrations of metam-sodium and MITC expressed as metam-sodium).

The biological results are reported based on geometric mean measured concentrations of total metam-sodium.

Inhibition of biomass and growth:

The 72 hour E_rC_{50} value based on growth rate was calculated to be 0.339 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.295 - 0.388 mg/L). The 72 hour E_rC_{10} value was calculated to be 0.0779 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0568 - 0.105 mg/L). The no

observed effect concentration (NOEC) value based on growth rate was determined to be 0.0813 mg/L (mean measured total metam-sodium).

The 72 h E_yC_{50} value based on yield was calculated to be 0.118 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0983 - 0.142 mg/L). The no observed effect concentration (NOEC) value based on yield was determined to be 0.0378 mg/L (mean measured total metam-sodium).

The 72 h E_bC_{50} value based on area under the curve was calculated to be 0.117 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0946 - 0.146 mg/L). The 72 h E_bC_{10} value was calculated to be 0.0482 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0225 - 0.0846 mg/L). The no observed effect concentration (NOEC) value based on area under the curve was determined to be 0.0378 mg/L (mean measured total metam-sodium).

The mean coefficient of variation (CoV) for daily growth rates in control cultures ranged between 2.0 and 4.7 during the definitive test and the CoV for the average specific growth rates of the control cultures was 1.3 during the 72 hour exposure period.

No microscopic abnormalities of the cells were detected.

A summary of algal growth rate, yield and area under the growth curve (AUC) is presented in the table below.

Table B.2.9.2.2-52: Measured concentrations of total metam-sodium in test media at test initiation (0 hours), 24 hours and at test termination (72 hours) of the Algal Growth Inhibition Test with *Pseudokirchneriella subcapitata*

Nominal concentrations		Measured concentrations (mg total metam-sodium/L) [b] Values in parentheses represent % of nominal concentration			
mg test item/L	**mg metam-sodium/L [a]**	**0 hours**	**24 hours**	**72 hours**	**Overall geometric mean**
control		n.d.	< LOQ	0.00716	-
de-oxygenated control		n.d.	< LOQ	0.00718	-
0.155	0.0693	0.0769 (111 %)	0.0417 (60 %)	0.0168 (24 %)	0.0378 (55 %)
0.155 *	0.0693*	-	-	0.0320 (46 %)	-
0.342	0.153	0.188 (123 %)	0.0900 (59 %)	0.0318 (21 %)	0.0813 (53 %)
0.751	0.336	0.351 (104 %)	0.153 (46 %)	0.0573 (17 %)	0.145 (43 %)
1.65	0.738	0.879 (119 %)	0.407 (55 %)	0.174 (24 %)	0.396 (54 %)
3.64	1.63	1.98 (121 %)	0.936 (57 %)	0.279 (17 %)	0.803 (49 %)
8.00	3.58	3.80 (106 %)	2.33 (65 %)	1.05 (29 %)	2.10 (59 %)
8.00 *	3.58*	-	-	1.07 (30 %)	-

[a] Based on 44.7 % w/w purity of test item

[b] Sum of the concentrations of metam-sodium and MITC expressed as metam-sodium

n.d. – None detected (< LOD of 0.005 mg/L for metam-sodium and 0.001 mg/L for MITC)

** Culture medium incubated under test conditions without algal cells*

Table B.2.9.2.2-53: Inhibtion of growth rate, yield and AUC of *Pseudokirchneriella subcapitata*, exposed to metam-sodium (technical grade) for 72 hours under static test conditions

Concentration (mg test item/L)		72 h growth rate		72 h yield		72 h area under curve	
Nominal	Mean measured	Mean	% inhibition	Mean	% inhibition	Mean	% inhibition
Controls		0.070	-	1530439	-	27.9	-
0.155	0.0378	0.068	3.0	1408867	7.9	24.1	13.5
0.342	0.0813	0.066	5.0	1204889 *	21.3	21.1 *	24.1
0.751	0.145	0.054 *	23.2	480211.3 *	68.6	8.9 *	67.9
1.65	0.396	0.030 *	56.6	89700.00 *	94.1	2.6 *	90.7
3.64	0.803	0.015 *	77.9	30911.00 *	98.0	0.6 *	97.7
8.00	2.10	0.006 *	90.8	17289.00 *	98.9	-0.1 *	100.3

n.d. – None detected (<LOD of 0.005 mg/L for metam sodium)
** Significant difference in comparison to the control; P<0.05 (William's test)*

Assessment and conclusions:

The effects of metam-sodium (technical grade) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* were assessed under static exposure conditions over a period of 72 hours in accordance with OECD test guideline 201 (2006).
The overall geometric mean measured concentrations of total metam-sodium were 0.0378, 0.0813, 0.145, 0.396, 0.803 and 2.10 mg/L (between 43 % and 59 % of nominal values). The biological results are reported based on mean measured concentrations of total metam-sodium (metam-sodium and its degradation product methyl isothiocyanate (MITC)).
The study was considered valid as all validity criteria were met. The 72 hour E_bC_{50} value for *Pseudokirchneriella subcapitata* was calculated to be 0.117 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0946 – 0.146 mg/L). The no observed effect concentration (NOEC) value based on area under the growth curve was determined to be 0.0378 mg/L (mean measured total metam-sodium).
The 72 hour E_rC_{50} value based on growth rate was calculated to be 0.339 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.295 - 0.388 mg/L). The 72 hour E_rC_{10} value was calculated to be 0.0779 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0568 - 0.105 mg/L). The no observed effect concentration (NOEC) value based on growth rate was determined to be 0.0813 mg/L (mean measured total metam-sodium).
The 72 hour E_yC_{50} value based on yield was calculated to be 0.118 mg/L (mean measured total metam-sodium) (95 % confidence limits: 0.0983 - 0.142 mg/L). The no observed effect concentration (NOEC) value based on yield was determined to be 0.0378 mg/L (mean measured total metam-sodium).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.118 mg total metam-sodium/L (mean measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.117 mg total metam-sodium/L (mean measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.339 mg total metam-sodium/L (mean measured)
Analytical method:
This study is submitted for information only and is not relied upon for risk assessment. Method validation is acceptable according to SANCO/3029/99 rev. 4.
Assessment and conclusion by Lainco:
The study is acceptable.

E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h, static) = 0.339 mg metam-sodium/L (mean measured total metam-sodium and MITC) (95 % confidence limits: 0.295 – 0.388 mg/L)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: factor of 120 for the control and factor of 122 for the de-oxygenated control)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 2.0 – 4.7 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 7 % (measured: 1.3 %)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/80 and 102 (Taminco) and KCA 4.1.2/15 (Lainco), for further details).
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.118 mg total metam-sodium/L (mean measured)
$NOEC_y$ (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.0378 mg total metam-sodium/L (mean measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.117 mg total metam-sodium/L (mean measured)
E_bC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.0482 mg total metam-sodium/L (mean measured)
$NOEC_b$ (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.0378 mg total metam-sodium/L (mean measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.339 mg total metam-sodium/L (mean measured)
E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.0779 mg total metam-sodium/L (mean measured)
$NOEC_r$ (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.0813 mg total metam-sodium/L (mean measured)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.1/04.

Data point:	KCA 8.2.6.1/04
Report author:	[redacted]
Report year:	2020b
Report title:	Metam sodium (technical grade): Algal growth inhibition assay - Statistical Re-analysis.
Report No.:	CEA.2142
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted:

	OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Due to some deviations compared to the current guideline as well as the fact that the test item is metam, which is not driving the risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report from Huntington Life Sciences, Study code LNO0008 ([redacted] 2011; KCA 8.2.6.1/03), for the toxicity of Metam Sodium on the growth of the green alga *Pseudokirchneriella subcapitata* did not provide values for the E_yC_{10}, E_yC_{20}, E_rC_{20} and E_bC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal concentrations of Metam Sodium at 0.155, 0.342, 0.751, 1.65, 3.64 and 8 mg/L as well as a control and a de-oxygenated control. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 106 % and 123 % of the nominal values at test initiation and reduced to 17 % and 29 % of the nominals by the end of the study. In line with the original study, the initial mean measured test concentrations were used for these re-analyses, the concentrations were: 0.0378, 0.0813, 0.145, 0.396, 0.803 and 2.1 mg/L. No significant differences were determined between the control and de-oxygenated control. However, in line with the original study, the results of the statistical re-analyses in this report are reported in comparison to the de-oxygenated control.

The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (μ)
- Biomass (b)

Statistical analyses of the available data for **yield** (Y) revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated.

Table B.2.9.2.2-54: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to metam-sodium for 7 days under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	24 hours		48 hours		72 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg/L]	0.034	0.061	0.027	0.043	0.061	0.076
lower 95 %-cl	0.020	0.042	0.017	0.030	0.034	0.051
upper 95 %-cl	0.048	0.080	0.036	0.053	0.077	0.090

cl: confidence limits

Statistical analyses of the available data for **growth rate** (μ) revealed that the following E_rC_{20} values were reliably calculated:

Table B.2.9.2.2-55: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to metam-sodium for 7 days under static test conditions, statistical re-analysis for growth rate, based on initial measured values

Parameter	24 hours	72 hours
	E_rC_{20}	E_rC_{20}
Value [mg/L]	0.150	0.134
lower 95 %-cl	0.109	0.108
upper 95 %-cl	0.187	0.158

cl: confidence limits

Statistical analyses of the available data for **biomass** (b) revealed that the following E_bC_{20} values were reliably calculated:

Table B.2.9.2.2-56: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to metam-sodium for 7 days under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours	48 hours	72 hours
	E_bC_{20}	E_bC_{20}	E_bC_{20}
Value [mg/L]	0.061	0.043	0.057
lower 95 %-cl	0.046	0.033	0.043
upper 95 %-cl	0.075	0.052	0.068

cl: confidence limits

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints from re-analysis:

Statistical analysis of the available data for yield (Y) revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_yC_{10} (*Pseudokircheneriella subcapitata*, 24 h) = 0.034 mg a.s./L (initial mean measured)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 48 h) = 0.027 mg a.s./L (initial mean measured)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 72 h) = 0.061 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 24 h) = 0.061 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 48 h) = 0.043 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.076 mg a.s./L (initial mean measured)

Statistical analysis of the available data for growth rate (μ) revealed that the following EC_{20} values were reliably calculated:

E_rC_{20} (*Pseudokircheneriella subcapitata*, 24 h) = 0.150 mg a.s./L (initial mean measured)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.134 mg a.s./L (initial mean measured)

Statistical analysis of the available data for biomass (b) revealed that the following EC_{20} values were reliably calculated:

E_bC_{20} (*Pseudokircheneriella subcapitata*, 24 h) = 0.061 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 48 h) = 0.043 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.057 mg a.s./L (initial mean measured)

Assessment and conclusion by Lainco:

The study is mentioned in the dossier of the applicant Lainco, however no statistical re-analysis is presented.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analysis of the available data for yield (Y) revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_yC_{10} (*Pseudokircheneriella subcapitata*, 24 h, static) = 0.034 mg a.s./L (initial mean measured)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 48 h, static) = 0.027 mg a.s./L (initial mean measured)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.061 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 24 h, static) = 0.061 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 48 h, static) = 0.043 mg a.s./L (initial mean measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.076 mg a.s./L (initial mean measured)
Statistical analysis of the available data for growth rate (μ) revealed that the following EC_{20} values were reliably calculated:
E_rC_{20} (*Pseudokircheneriella subcapitata*, 24 h, static) = 0.150 mg a.s./L (initial mean measured)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.134 mg a.s./L (initial mean measured)
Statistical analysis of the available data for biomass (b) revealed that the following EC_{20} values were reliably calculated:
E_bC_{20} (*Pseudokircheneriella subcapitata*, 24 h, static) = 0.061 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 48 h, static) = 0.043 mg a.s./L (initial mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.057 mg a.s./L (initial mean measured)
Since the test item is metam-sodium, which is not driving the aquatic risk assessment, this study is considered acceptable as supplementary data and therefore not relied upon.

Studies with MITC

Data point:	KCA 8.2.6.1/05
Report author:	[redacted]
Report year:	1998
Report title:	Effect of Methyl isothiocyanate on the Growth of the Green Alga *Pseudokirchneriella subcapitata*.
Report No.:	48881
Document No.:	98/10767
Guidelines followed in study:	OECD Guideline 201
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

The effects of methyl isothiocyanate (MITC) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated according to OECD guideline 201.

The 72 hours test was conducted with 5 replicates at each concentration and the control (10 for the solvent control), initial cell densities of 1 x 10^4 cells/mL and nominal concentrations of 0.03, 0.06, 0.1, 0.19, 0.35, 0.65 and 1.2 mg MITC/L.
Analytical verification of test substance concentrations was carried out in each concentration at the beginning and at the end of the test. The test substance was not stable in water. Analytical measurements yielded accordingly only 58 % - 74 % of nominal in samples from test initiation and 0 % - 13 % in samples from the end of the test. The biological results were based on initial measured test concentrations. No morphological effects were observed. The following results with respect to algal growth rates "r" and biomass "b" were obtained using probit calculations:
E_rC_{50} (0 – 72 h) = 0.58 mg MITC/L (95 % confidence limits: 0.43 - 0.79 mg/L)
E_rC_{10} (0 – 72 h) = 0.19 mg MITC/L (95 % confidence limits: 0.16 - 0.23 mg/L)
E_bC_{50} (0 – 72 h) = 0.28 mg MITC/L (95 % confidence limits: 0.20 - 0.72 mg/L)
E_bC_{10} (0 – 72 h) = 0.10 mg MITC/L (95 % confidence limits: 0.03 - 0.15 mg/L)

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 80525, chemical purity: 99.0 %
Test species:	Green algae (*Pseudokirchneriella subcapitata*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control: acetone), 0.03, 0.06, 0.10, 0.19, 0.35, 0.65 and 1.2 mg MITC/L
Dilution medium:	AAP algal medium
Initial cell density, number of replicates:	1 x 10^4 cells/mL, 5 replicates for the control, 10 replicates for the solvent control and 5 replicates per treatment group
Time of exposure:	72 hours In-life dates: August 10th to 13th 1998
Test conditions:	temperature: 22 – 23 °C pH: 8.0 at start, 7.82 – 8.03 at end photoperiod: continuous illumination light intensity: 8000 lux
Test procedure:	The effects of methyl isothiocyanate (MITC) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated according to OECD guideline 201. Nominal MITC concentrations of 0.03, 0.06, 0.1, 0.19, 0.35, 0.65 and 1.2 mg MITC/L were tested. Five replicates were conducted at test concentrations and the standard control. The solvent control was run with 10 replicates. Despite a fairly high water solubility of the test substance, acetone was added as solvent because otherwise the process of solubilisation was very slow. 40 mg of the test substance was dissolved in 2 mL acetone. 0.5 mL of this solution was added to 1 L OECD nutrient solution. This stock solution was further diluted with nutrient solution containing the same amount of acetone (0.06 mL/L) to reach the desired test concentrations. Flasks were inoculated with algae from a pre-culture to get an initial cell concentration of about 1 x 10^4 cells/mL. The culture flasks were placed in a temperature controlled incubator until sampling. During the experiment the algae were kept in suspension by constant shaking at about 135 rpm.

Test item analysis: At the beginning and at the end of the test samples were taken for verification of test substance concentrations. MITC was quantified by reversed phase HPLC using UV-detection at the wavelength of 248 nm.

Observations: Cells concentration in each flask was determined 24, 48 and 72 hours after starting the experiment with a spectrophotometer (623 nm, 5 cm glass cuvettes). To obtain the actual number of cells/mL a linear correlation (calibration curve) was calculated from the cell numbers (counted under a microscope) versus extinction values.
At the last sampling interval, the pH of all individual samples (controls as well as treated samples) was measured.

Statistical evaluation: At each sampling interval, one aliquot of each of the replicates of test and control flasks was collected for evaluation of algal densities. Subsequently the mean values per treatment were calculated and growth curves plotted. In order to determine the concentration/effect-relationship, the area below the growth curves was calculated.
The percent inhibition of the cell growth (biomass) at each test concentration is calculated from the difference between the area under the control growth curve and the area under the growth curve at each test concentration. Growth rate is calculated from the difference between the average growth rate of the controls and the average growth rates at the test concentrations.
The % inhibition results for biomass or growth rates are used to determine the $EC_{50/10}$. The mathematical determination of the EC_{50} was done by probit analysis according to a method described in: "E. Weber, Grundriss der biologischen Statistik, Fischer Verlag, Jena, 1986" and in: "D.J. Finney, Probit Analysis, Cambridge University Press, 1952".

Findings:

Analytical results: The test substance is not stable in water. Analytical measurements yielded accordingly only 58 - 74 % of nominal in samples from test initiation and 0 - 13 % in samples from the end of the test. The biological results were based on initial measured test concentrations.

Inhibition of biomass and growth: After 72 hours of exposure, inhibition of biomass relative to the control in the 0.019, 0.041, 0.070, 0.134, 0.207, 0.384 and 0.692 mg MITC/L treatment groups was 0.1 %, 0 %, 9.9 %, 10.8 %, 27.3 %, 71.9 % and 100 %, respectively. Inhibition of growth rate relative to the control was 0.1 %, -0.1 %, 2.1 %, 2.0 %, 8.2 %, 34.1 % and 100 %, respectively. Detailed results on biomass and cell growth are presented in the tables below.

Table B.2.9.2.2-57: Measured concentrations of MITC in exposure algal medium at test initiation (0 hours) and at test termination (72 hours) of the Algal Growth Inhibition Test with *Pseudokirchneriella subcapitata*

Nominal concentration (mg MITC/L)	Mean measured concentrations (mean of two samples) (mg MITC/L)					% of nominal concentration
	0 hours	% of nominal	72 hours	% of nominal	Mean	
Control	n.d.	-	-	--	-	-
0.03	0.02	63.3	n.d.[1]	0	0.0113	37.5
0.06	0.04	67.8	n.d.[1]	0	0.0213	35.4
0.10	0.07	74.2	n.d.[1]	0	0.0363	36.3
0.19	0.13	70.4	n.d.[1]	0	0.1263	66.4
0.35	0.21	59.1	0.04	11.3	0.125	35.7
0.65	0.38	59.1	0.09	13.4	0.255	39.2
1.20	0.69	57.7	n.d.[1]	0	0.3563	29.7

n.d.: not detected

[1] Half LOQ (0.0025 mg/L) was used for geometric mean calculation

Table B.2.9.2.2-58: Mean cell numbers and percent inhibition in biomass of *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions

Day 0 measured test concentrations (mg MITC/L)	24 hours	48 hours	72 hours	% Inhibition in biomass
	Mean cell number (cells/mL)	Mean cell number (cells/mL)	Mean cell number (cells/mL)	
control	56570	290500	1078000	-
solvent control	59310	292500	1115000	-
0.019	60950	294100	1107000	0.1
0.041	58390	291200	1119000	0
0.070	54740	260900	1012000	9.9
0.134	52180	254500	1010000	10.8
0.207	44870	216600	812700	27.3
0.384	30990	69720	345600	71.9
0.692	13810	6866	10060	100

Table B.2.9.2.2-59: Mean growth rate per day and percent inhibition of *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions

Day 0 measured test concentrations (mg MITC/L)	24 hours	48 hours	72 hours	Average 0-72 h	% Inhibition
	Mean growth rate (per day)	Mean growth rate (per day)	Mean growth rate (per day)		
control	1.730	1.637	1.312	1.560	-
solvent control	1.779	1.594	1.337	1.570	
0.019	1.807	1.574	1.325	1.569	0.1
0.041	1.764	1.606	1.346	1.572	-0.1
0.070	1.699	1.558	1.356	1.538	2.1
0.134	1.648	1.543	1.380	1.539	2.0
0.207	1.497	1.572	1.258	1.442	8.2
0.384	1.103	0.721	1.278	1.034	34.1
0.692	0.299	-1.112	0.681	-0.075	100

Assessment and conclusions:

The effects of methyl isothiocyanate (MITC) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated according to OECD guideline 201**.** The following results with respect to algal growth rates "r" and biomass "b" were obtained:

E_rC_{50} (0 – 72 h) = 0.58 mg MITC/L (95 % confidence limits: 0.43 - 0.79 mg/L)
E_rC_{10} (0 – 72 h) = 0.19 mg MITC/L (95 % confidence limits: 0.16 - 0.23 mg/L)
E_bC_{50} (0 – 72 h) = 0.28 mg MITC/L (95 % confidence limits: 0.20 - 0.72 mg/L)
E_bC_{10} (0 – 72 h) = 0.10 mg MITC/L (95 % confidence limits: 0.03 - 0.15 mg/L)

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.28 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.58 mg MITC/L (initial measured)
Analytical method:
Method validation not fully in line with the guidance SANCO/3029/99 rev. 4 on analytical validation but given that the endpoint is in line with state of the art studies, this is considered to be fit for purpose.
This study is not taken into account for the endpoint derivation.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: > 16)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 7 %

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
No further information on the analytical method used.
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.28 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.58 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.1/06.

Data point:	KCA 8.2.6.1/06
Report author:	[redacted]
Report year:	2019a
Report title:	Effect of Methyl isothiocyanate on the Growth of the Green Alga *Pseudokirchneriella subcapitata* – Statistical Re-analysis
Report No.:	CEA.2034
Document No.:	-

Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from BASF Aktiengesellschaft, study code 48881 ([redacted] 1998; KCA 8.2.6.1/05) for the toxicity of Methyl isothiocyanate on the growth of the freshwater alga *Pseudokirchneriella subcapitata* did not provide estimates of the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.
The test used nominal concentrations of Methyl isothiocyanate at 0.03, 0.06, 0.10, 0.19, 0.35, 0.65 and 1.2 mg/L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 58 % to 74 % of the nominal values from test initiation and 0 % to 13 % in samples at the end of the test. In line with the original study, the initial measured test concentrations were used for this analysis, the concentrations were 0.019, 0.041, 0.070, 0.134, 0.207, 0.384 and 0.692 mg/L. In addition, no statistically significant differences were determined between the control data and solvent control, therefore all analyses reported here used pooled control data.

The following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)
- Biomass (b)

Statistical analyses of the available data for **yield** revealed that the following E_yC_{10}, E_yC_{20} and E_yC_{50} values were reliably calculated:

Table B.2.9.2.2-60: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 7 days under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	24 hours			48 hours			72 hours		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg a.i./L]	0.126	0.172	0.314	0.143	0.177	0.266	0.145	0.184	0.288
lower 95 %-cl	0.098	0.144	0.284	0.118	0.154	0.246	0.096	0.136	0.248
upper 95 %-cl	0.150	0.197	0.349	0.162	0.195	0.289	0.180	0.218	0.336

cl: confidence limits

Statistical analyses of the available data for average specific **growth rate** revealed that the following E_rC_{10}, E_rC_{20} and E_rC_{50} values were reliably calculated:

Table B.2.9.2.2-61: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 7 days under static test conditions, statistical re-analysis for growth rate, based on initial measured values

Parameter	24 hours			48 hours			72 hours		
	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg a.i./L]	0.197	0.257	0.427	0.235	0.280	0.391	0.292	0.334	0.432
lower 95 %-cl	0.157	0.218	0.391	0.194	0.245	0.367	0.220	0.278	0.401
upper 95 %-cl	0.230	0.289	0.468	0.264	0.306	0.417	0.329	0.365	0.485

cl: confidence limits

Statistical analyses of the available data for **biomass** revealed that the following EC_{10}, EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.2-62: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 7 days under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours			72 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [mg a.i./L]	0.126	0.172	0.314	0.143	0.181	0.281
lower 95 %-cl	0.090	0.135	0.275	0.092	0.132	0.241
upper 95 %-cl	0.156	0.203	0.361	0.178	0.215	0.331

cl: confidence limits

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Original Endpoints:
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.28 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.58 mg MITC/L (initial measured)
Endpoints from re-analysis:
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.281 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.432 mg MITC/L (initial measured)
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.281 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.432 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.1/07
Report author:	[redacted]
Report year:	2012d
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*).
Report No.:	-
Document No.:	703A-106

Guidelines followed in study:	OECD 201 (2006) EU Directive 92/69/EEC, Method C.3. US EPA OPPTS Number 850.5400 (draft, 1996) ISO 14442 Standard (2006)
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	No, not previously submitted at EU level
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

In a static acute toxicity test, the freshwater green alga, *Pseudokirchneriella subcapitata*, was exposed for 96 hours to 6 nominal concentrations ranging between 0.051 and 5.0 mg methyl isothiocyanate (MITC)/L and a negative control. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL. Measured concentrations declined by day 4, and ranged from 12 % to 65 % of nominal. The results of the study are based on day 0 measured concentrations of 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L, representing 86 %, 81 %, 78 %, 76 %, 72 % and 74 % of the target nominal test concentrations.
The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 0.21 (0.14 to 0.33) mg a.s./L and 0.28 (0.17 to 0.45) mg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 0.12 (0.092 to 0.15) mg a.s./L and 0.15 (0.073 to 0.23) mg a.s./L, respectively. The 72 and 96 hour no-observed-adverse-effect-concentrations (NOAEC), based on effects on yield and growth rate were 0.044 and 0.11 mg a.s./L, respectively.

Materials and methods:

Test substance:	Methylisothiocyanate (MITC), batch no.: 56198PJV, chemical purity: 99.7 % w/w
Test species:	Green algae (*Pseudokirchneriella subcapitata*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 0.051, 0.13, 0.32, 0.80, 2.0 and 5.0 mg MITC/L
Dilution medium:	OECD Algal Nutrient Medium
Initial cell density, number of replicates:	5000 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	96 hours In-life dates: September 15th to 19th 2011
Test conditions:	temperature: 23.3 – 23.9 °C pH: 7.5 – 7.8 at start, 8.0 – 10.8 at end photoperiod: continuous illumination light intensity: 4290 – 5020 lux
Test procedure:	The freshwater green alga, *Pseudokirchneriella subcapitata*, was exposed for 96 hours to nominal concentrations of 0, 0.051, 0.13, 0.32, 0.80, 2.0 and 5.0 mg a.s./L. Three replicate test chambers were maintained in each treatment group and six replicates were

	maintained in the control group. A single abiotic replicate was maintained at the highest test concentration to assess stability of the test substance under test conditions. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL.
Test item analysis:	Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group prior to distribution into the test chambers. At exposure termination, samples were collected from the pooled replicates from each treatment and control group. The abiotic sample was collected from replicate D of the 5.0 mg a.s./L treatment group. Samples were collected into glass scintillation vials and acidified with two drops of 10 % phosphoric acid prior to analysis. The analytical method consisted of extracting the samples with diethyl ether. An aliquot of each diethyl ether phase was transferred to autosampler vials and submitted for analysis by gas chromatography with mass selective detection (GC-MS).
Observations:	Test medium samples were collected from each replicate of the treatment and control groups for the determination of algal cell densities. Samples were collected at approximately 24 hour intervals during the 96 hour exposure using a syringe and were held for a maximum of three days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts were performed using an electronic particle counter (Coulter Electronics, Inc.). Cell counts for samples collected during the test were conducted once instrument linearity was demonstrated (i.e., the R-squared value obtained through the regression analysis was at least 0.99957). A single aliquot of each sample collected during the test was diluted with an electrolyte solution (Isoton®). Three 0.5-mL volumes of the diluted sample were counted, and the resulting counts were averaged. The cell density of the sample was determined by adjusting the mean cell count (cells/mL) obtained using the particle counter, based upon the Y-intercept and slope calculated through the regression analysis, and the dilution factor. Samples of test solution were collected from each replicate at the end of the test. These samples were pooled within their respective treatments, and sub-samples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregation or flocculation of cells, and adherence of the cells to the test chamber.
Statistical evaluation:	The calculation of cell densities, yield, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using “The SAS System for Windows, Version 8.2”. Growth rate was calculated for each replicate of the control and treatment groups. Yield was calculated for each replicate of the control and treatment groups as the final biomass (cell density) in the exposure period minus the initial biomass (cell density). Inhibition values were calculated for each treatment group as the percent reduction

in yield and growth rate relative to the negative control replicates for each 24 hour interval. The E_yC_{50} and E_rC_{50} values and their 95 % confidence intervals were calculated, when possible, using non-linear regression with replicate data (yield or growth rate) and day 0 measured test concentrations. Non-linear regression analyses were conducted based on the measured MITC concentrations in samples collected at test initiation (hour 0). Non-linear regression failed to produce an EC_{50} value for yield (E_yC_{50}) at 96 hours. Non-linear regression generated an EC_{50} for yield at 72 hours of 0.11 µg a.s./L, but failed to produce realistic 95 % confidence intervals (< 0.044 to > 3.7 µg a.s./L). The Schabenberger Hormetic Logistic model was used to estimate the 72 and 96 hour E_yC_{50} values and their corresponding 95 % confidence intervals. The 24, 72 and 96 hour yield and growth rate data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro-Wilk's and Levene's tests, respectively. The 24, 72 and 96 hour yield data failed to meet assumptions for normality. Log transformation (natural log) of the data resolved these issues regarding normal distribution. The treatment groups were compared to the negative control using Dunnett's one-tailed t-test ($\alpha = 0.05$). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 and 96 hours.

Findings:

Analytical results:

Measured concentrations on day 0 ranged from 72 to 86 % of nominal. Measured concentrations declined by day 4, and ranged from 12 to 65 % of nominal. The results of the study are based on day 0 measured concentrations of 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L, representing 86, 81, 78, 76, 72 and 74 % of the target nominal test concentrations. The measured concentration in the abiotic control was 74 % of nominal at test initiation and 65 % of nominal at test termination.

Inhibition of biomass and growth:

Growth in the control replicates decreased from 72 to 96 hours. This decrease is probably due to the maximum cell density being achieved in the test system after 72 hours. Exponential growth, characterized by the linear section of the growth curve, occurred from 0 to 72 hours.

After 72 hours of exposure, inhibition of yield relative to the negative control in the 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups was 4 %, 35 %, 95 %, 99 %, 100 % and 100 %, respectively. Inhibition of growth rate relative to the negative control in the 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups was 1 %, 9 %, 59 %, 85 %, 91 % and 93 %, respectively. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups. Consequently, the 72 hour NOAEC for yield and growth rate was 0.044 mg a.s./L.

After 96 hours of exposure, inhibition of yield relative to the negative control in the 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups was -19 %, -3 %, 93 %, 98 %, 99 % and 99 %, respectively. Inhibition of growth rate relative to the negative control in the 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups was -4 %, -1 %, 54 %, 79 %, 85 % and 88 %, respectively. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups. Consequently, the 96 hour NOAEC for yield and growth rate was 0.11 mg a.s./L.

After 96 hours of exposure, there were aggregations of cells in the negative control and the 0.044 and 0.11 mg a.s./L treatment groups. Adherence of cells to the test chambers was noted in the negative control and the 0.044 and 0.11 mg a.s./L treatment groups. Cells in the 0.25, 0.61, 1.4 and 3.7 mg a.s./L treatment groups appeared enlarged when compared to cell in the negative control, during the microscopic examinations of the cells. Enlargement of cells is considered to be a treatment related effect on freshwater alga. Adherence of cells to the test chamber and aggregation of cells is commonly observed in studies conducted using a closed-system design and are not considered to be treatment related because these observations were noted in the negative control group.

Table B.2.9.2.2-63: Measured concentrations of MITC in algal medium samples at test initiation (day 0) and at test termination (day 4) of the Algal Growth Inhibition Test with *Pseudokirchneriella subcapitata*

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)			Mean	% of nominal concentration
	Day 0	Day 4	% of day 0		
control	< LOQ[1]	< LOQ[1]	-	-	-
0.051	0.0440	0.00605*	13.8	0.025	49.1
0.13	0.106	< LOQ	-	0.0592[2]	45.6
0.32	0.249	0.203	81.5	0.226	70.6
0.80	0.609	0.513	84.2	0.561	70.1
2.0	1.44	1.25	86.8	1.35	67.3
5.0	3.71	2.96	79.8	3.34	66.7

[1] The limit of quantitation (LOQ) was 0.0250 mg a.s./L, calculated as the product of the concentration of the lowest calibration standard (0.0500 mg a.s./L) and the dilution factor of the matrix blank samples (0.500). The limit of detection (LOD) was 0.00131 mg a.s./L.

[2] Half LOQ (0.0125 mg a.s./L) was used for mean calculation.

Table B.2.9.2.2-64: Mean yield and percent inhibition of *Pseudokirchneriella subcapitata*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentrations (µg a.s./L)	24 hours[1]		48 hours[1]		72 hours[1]		96 hours[1]	
	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]
negative control	30698	--	170390	--	662201	--	544111	--
0.044	29898	3	168146	1	638695	4	644906	-19
0.11	26248	14	147492	13	427478*	35	561112	-3
0.25	14313	53	29584	83	32758*	95	38942*	93
0.61	3595	88	4358	97	5512*	99	8234*	98
1.4	2118	93	2543	99	2691*	100	4963*	99
3.7	1467	95	1811	99	2208*	100	3718*	99

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.
[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.
** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Table B.2.9.2.2-65: Mean growth rate (per hour) and percent inhibition of *Pseudokirchneriella subcapitata*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentrations (µg a.s./L)	24 h[1]		48 h[1]		72 h[1]		96 h[1]	
	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]
negative control	0.0813	--	0.0740	--	0.0679	--	0.0488	--
0.044	0.0809	0	0.0738	0	0.0675	1	0.0507	-4
0.11	0.0763	6	0.0712	4	0.0619*	9	0.0492	-1
0.25	0.0556	32	0.0402	46	0.0279*	59	0.0226*	54
0.61	0.0226	72	0.0130	82	0.0103*	85	0.0101*	79
1.4	0.0147	82	0.0086	88	0.0060*	91	0.0072*	85
3.7	0.0107	87	0.0064	91	0.0050*	93	0.0057*	88

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.
[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.
** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Assessment and conclusions:

The freshwater green alga, *Pseudokirchneriella subcapitata*, was exposed to a geometric series of six concentrations of MITC ranging from 0.044 to 3.7 mg a.s./L, based on measured concentrations of MITC at test initiation (day 0). Effects were evaluated based on yield and growth rate. The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 0.21 (0.14 to 0.33) mg a.s./L and 0.28 (0.17 to 0.45) mg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 0.12 (0.092 to 0.15) mg a.s./L and 0.15 (0.073 to 0.23) mg a.s./L, respectively. The 72 and 96 hour no-observed-adverse-effect-concentrations (NOAEC), based on effects on yield and growth rate were 0.044 and 0.11 mg a.s./L, respectively.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.

Endpoints:
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.12 mg MITC/L (initial measured)
E_yC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 0.15 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.21 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 0.28 mg MITC/L (initial measured)
Analytical method:
Method validation not fully acceptable according to SANCO/3029/99 rev. 4 but is fit for purpose.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: factor of 133)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 21.9 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 7 % (measured: 3.42 %)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/58, for further details).
The study is relied upon, though the endpoint is not critical.
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.12 mg MITC/L (initial measured)
E_yC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.15 mg MITC/L (initial measured)
$NOAEC_y$ (*Pseudokircheneriella subcapitata*, 72 h and 96 h, static) = 0.044 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.21 mg MITC/L (initial measured)
E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.28 mg MITC/L (initial measured)
$NOAEC_r$ (*Pseudokircheneriella subcapitata*, 72 h and 96 h, static) = 0.11 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.1/08.

Data point:	KCA 8.2.6.1/08
Report author:	[redacted]
Report year:	2020c
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*) – Statistical Re-analysis.
Report No.:	CEA.2140
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted:

	OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from Wildlife International, Ltd., Study code 703A-106 ([redacted], 2012; KCA 8.2.6.1/07), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the green alga *Pseudokirchneriella subcapitata* did not provide values for the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. In addition, the biomass integral over the total growth curve (area under the growth curve) was not calculated as part of the original study and estimates of the EC_x values were not provided. Consequently, the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal concentrations of MITC at 0.051, 0.13, 0.32, 0.80, 2.0 and 5.0 mg a.s./L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 72 % to 86 % of the nominal values from test initiation and reduced to 12 % to 65 % of the nominals by the end of the study. In line with the original study, the initial measured test concentrations were used for these re-analyses, the concentrations were 0.044, 0.11, 0.25, 0.61, 1.4 and 3.7 mg a.s./L.
The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (μ)
- Biomass (b)

Statistical re-analysis of the available data for **yield** revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table B.2.9.2.2-66: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	24 hours		48 hours		72 hours		96 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg/L]	0.09	0.12	0.10	0.12	0.08	0.09	0.12	0.13
lower 95 %-cl	0.07	0.10	0.09	0.11	0.07	0.09	0.11	0.13
upper 95 %-cl	0.10	0.14	0.11	0.13	0.08	0.09	0.13	0.14

cl: confidence limits

Statistical re-analysis of the available data for **growth rate** revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Table B.2.9.2.2-67: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for growth rate, based on initial measured values

Parameter	24 hours		48 hours		72 hours		96 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [mg/L]	0.10	0.17	0.11	0.15	0.09	0.13	0.10	0.14
lower 95 %-cl	0.08	0.14	0.10	0.15	0.09	0.12	0.09	0.14
upper 95 %-cl	0.13	0.19	0.12	0.16	0.10	0.13	0.11	0.15

cl: confidence limits

Statistical re-analysis of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-68: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours			48 hours			72 hours			96 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [mg/L]	0.09	0.12	0.24	0.10	0.12	0.18	0.08	0.10	0.15	0.09	0.11	0.15
lower 95 %-cl	0.07	0.11	0.22	0.09	0.12	0.18	0.08	0.10	0.14	0.09	0.10	0.14
upper 95 %-cl	0.10	0.14	0.26	0.11	0.13	0.19	0.08	0.10	0.15	0.09	0.11	0.15

cl: confidence limits

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Original Endpoints:

E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.12 mg MITC/L (initial measured)

E_yC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 0.15 mg MITC/L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 0.21 mg MITC/L (initial measured)

E_rC_{50} (*Pseudokircheneriella subcapitata*, 96 h) = 0.28 mg MITC/L (initial measured)

Endpoints from re-analysis:

Statistical analysis of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_yC_{10} (*Pseudokircheneriella subcapitata*, 72 h) = 0.08 mg MITC/L (initial measured)

E_yC_{10} (*Pseudokircheneriella subcapitata*, 96 h) = 0.12 mg MITC/L (initial measured)

E_yC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.09 mg MITC/L (initial measured)

E_yC_{20} (*Pseudokircheneriella subcapitata*, 96 h) = 0.13 mg MITC/L (initial measured)

Statistical analysis of the available data for growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h) = 0.09 mg MITC/L (initial measured)

E_rC_{10} (*Pseudokircheneriella subcapitata*, 96 h) = 0.10 mg MITC/L (initial measured)

E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.13 mg MITC/L (initial measured)

E_rC_{20} (*Pseudokircheneriella subcapitata*, 96 h) = 0.14 mg MITC/L (initial measured)

Statistical analysis of the available data for biomass revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_bC_{10} (*Pseudokircheneriella subcapitata*, 72 h) = 0.08 mg MITC/L (initial measured)

E_bC_{10} (*Pseudokircheneriella subcapitata*, 96 h) = 0.09 mg MITC/L (initial measured)

E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 0.10 mg MITC/L (initial measured)

E_bC_{20} (*Pseudokircheneriella subcapitata*, 96 h) = 0.11 mg MITC/L (initial measured)

Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analysis of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_yC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.08 mg MITC/L (initial measured)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.12 mg MITC/L (initial measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.09 mg MITC/L (initial measured)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.13 mg MITC/L (initial measured)
Statistical analysis of the available data for growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.09 mg MITC/L (initial measured)
E_rC_{10} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.10 mg MITC/L (initial measured)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.13 mg MITC/L (initial measured)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.14 mg MITC/L (initial measured)
Statistical analysis of the available data for biomass revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_bC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.08 mg MITC/L (initial measured)
E_bC_{10} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.09 mg MITC/L (initial measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 0.10 mg MITC/L (initial measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 96 h, static) = 0.11 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.1/09
Report author:	[redacted]
Report year:	2018b
Report title:	Methyl isothiocyanate (MITC) - Effect on *Pseudokirchneriella subcapitata* in a 72-Hour Algal Growth Inhibition Test.
Report No.:	IES Study 20180073
Document No.:	-
Guidelines followed in study:	OECD 201 (2011) Method C.3 of Commission Regulation (EU) No. 2016/266
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	No, not previously submitted at EU level
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities (exception: range-finding tests and pre-test for verification of the stability of the test item)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: Kanesho Soil Treatment, letter of access by Taminco is included, study may be used by Taminco in Europe only) (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

The impact of the test item Methyl isothiocyanate (MITC) on the growth of the freshwater green algal species *Pseudokirchneriella subcapitata* was investigated in a 72 hour static test.
The nominal test item concentrations tested were 14, 28, 56, 113, 225 and 450 µg/L. A control (test water without test item) was tested in parallel.
As the test item is a volatile substance, the test was performed using glass stoppered Erlenmeyer flasks completely filled with test medium, minimizing the air-space in the flasks and to avoid losses of test item by evaporation.
Due to the decrease of Methyl isothiocyanate (MITC) concentrations during the test period, the mean measured concentrations were calculated as the time-weighted geometric mean of the concentrations measured at all sampling dates (0, 24, 48 and 72 hours).
The NOEC and EC_{50} values based on growth rate and yield were 19 and 189 µg/L (growth rate) and 19 and 91 µg/L (yield), respectively.

Materials and methods:

Test substance:	Methylisothiocyanate (MITC), batch no.: STBB1308V, chemical purity: 99.6 %
Test species:	Green algae (*Pseudokirchneriella subcapitata*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 10, 29, 84, 244, 707 and 2051 µg MITC/L
Dilution medium:	AAP algal medium
Initial cell density, number of replicates:	5000 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	72 hours
Test conditions:	temperature: 23 °C pH: 7.0 at start, 7.3 at end photoperiod: continuous illumination light intensity: 73 – 76 µE s^{-1} m^{-2}
Test procedure:	The test organism used for the study was *Pseudokirchneriella subcapitata*. Based on the results of a range-finding test, following nominal test concentrations were tested: 14, 28, 56, 113, 225 and 450 µg MITC/L. A control (test water without test item) was tested in parallel. The test design included three replicates per test concentration and six replicates for the control. Additionally, two replicates per treatment were prepared for analytical samples at 24 and 48 hours, which were incubated in parallel to the test. The test was started using a nominal algal cell density of 5000 cells/mL. A static test design was applied. The duration of the test was 72 hours.
Test item analysis:	For measurement of the actual concentrations of the test item, duplicate samples without algae were taken from the test media of all test concentrations and the control at the start of the test. About 20 g of sodium chloride were added to the test samples subsequently to sampling. Then, the samples (50 mL) were extracted with 10 mL of internal standard solution. The organic phase was separated and frozen (at about -20 °C) immediately after extraction. The maximum storage time of the extracts until analysis was three days.

	The concentrations of the test item Methyl isothiocyanate (MITC) were determined in one of the duplicate samples from the control and all concentrations from all sampling dates. From the concentrations measured at all sampling dates, the mean measured concentrations were calculated. For this, the time-weighted geometric mean was used, as a decrease of test item concentrations during the test period was observed. Samples were analysed by gas chromatography with mass spectrometric detection (GC/MS).
Observations:	A small volume (100 µL per sampling) of the algal suspension was withdrawn daily from each test flask for the measurement of the biomass, and was not replaced. The algal biomass in the samples was determined by fluorescence measurement. The measurements were performed at least in duplicate at an excitation of 440 nm and emission of 680 nm. At the end of the test, a sample was taken from the control and from the nominal test concentration of 113 µg MITC/L to determine a potential influence of the test item on the algal cells. The shape and size of the algal cells were visually inspected. This test concentration was chosen because the algal cell density at the two highest nominal concentrations of 225 and 450 µg MITC/L was too low for a reliable examination. The experimental conditions were monitored. The light intensity was measured at the start of the test. The temperature in the incubator was monitored and recorded continuously. The pH was measured and recorded in each treatment at the start and end of the test. The appearance of the test media was visually controlled and recorded daily.
Statistical evaluation:	The 72 hour EC_{10}, EC_{20} and EC_{50} values for the inhibition of average growth rate and yield and their 95 % confidence intervals were calculated by Probit Analysis using linear maximum likelihood regression. For the determination of the LOEC and NOEC, the average growth rate and yield at the test concentrations were compared to the control values by Williams' t-test. Statistical analysis was performed using ToxRat Professional®.

Findings:

Analytical results:	Due to the decrease of Methyl isothiocyanate (MITC) concentrations during the test period, the mean measured concentrations were calculated as the time-weighted geometric mean of the concentrations measured at all sampling dates (0, 24, 48 and 72 hours). The biological results were related to the mean measured concentrations.
Inhibition of biomass and growth:	The test item had a significant inhibitory effect on the growth rate and on yield of the algae within the test period of 72 hours at the mean measured concentration of 37 µg MITC/L and at all higher test concentrations (results of Williams' t-test, one-sided smaller, $\alpha = 0.05$).

The 72 NOEC based on the growth rate and on yield was determined to be 19 µg MITC/L, since up to and including this test concentration the growth rate and yield of the algae were not significantly lower than in the control.
The 72 hour LOEC was determined to be 37 µg MITC/L due to the statistically significant reduction of the growth rate (µ) and the yield (Y) at this test concentration.
The 72 hour E_rC_{10} and E_rC_{20} were calculated to be 76 µg MITC/L (95 % confidence limits: 75 – 78 µg/L) and 104 µg MITC/L (95 % confidence limits: 103 – 106 µg/L), respectively.
The 72 hour E_rC_{50} was calculated to be 189 µg MITC/L (95 % confidence limits: 187 – 191 µg/L).
The 72 hour E_yC_{10} and E_yC_{20} were calculated to be 51 µg MITC/L (95 % confidence limits: 48 – 53 µg/L) and 62 µg MITC/L (95 % confidence limits: 60 – 64 µg/L), respectively.
The 72 hour E_yC_{50} for 72 hours was determined to be 91 µg MITC/L (95 % confidence limits: 88 – 93 µg/L).
The microscopic examination of the algal cells at the end of the test showed no difference between the algae growing at the nominal test concentration of 113 µg MITC/L and the algal cells in the control. The shape and size of the algal cells were obviously not affected by the test item up to at least this test concentration.
No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the test period.

Table B.2.9.2.2-69: Daily measured concentrations of MITC in the test media of the Algal Growth Inhibition Test with *Pseudokirchneriella subcapitata*

Nominal Test Item Concentration [µg/L]	Analytically Measured Concentration of the Test Item [µg/L]				Mean Measured Concentration (Geometric Mean) [µg/L]
	0 hours	24 hours	48 hours	72 hours	
14	9.90	9.49	9.13	7.31	9.0
28	20.1	19.1	19.0	16.0	19
56	39.9	38.4	37.0	31.7	37
113	79.5	75.8	74.6	66.5	74
225	158	152	146	142	149
450	320	314	310	296	311

Table B.2.9.2.2-70: Effect on Biomass of algae of *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Mean Measured Test Item Concentration [µg/L]	Biomass of Algae* (mean ± SD)		
		24 hours	48 hours	72 hours
Control	---	5.8 ± 0.89	41 ± 1.8	221 ± 12
14	9.0	6.1 ± 0.70	40 ± 0.26	221 ± 11
28	19	5.8 ± 0.57	38 ± 0.66	211 ± 7.5
56	37	5.6 ± 0.48	34 ± 0.38	194 ± 5.2
113	74	5.3 ± 0.36	29 ± 2.55	156 ± 3.6

225	149	4.0 ± 0.20	11 ± 0.92	25 ± 1.1
450	311	3.0 ± 0.20	3.4 ± 0.37	3.5 ± 0.13

** The biomass was determined by fluorescence measurement (mean of duplicate measurements per replicate) and is given as relative fluorescence units (x 10^4). At the start of the test, the initial cell density was 5000 algal cells/mL, corresponding to 0.85 x 10^4 relative fluorescence units.*

Table B.2.9.2.2-71: Effect on Average growth rates (µ) and percent inhibition for *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Mean Measured Test Item Concentration [µg/L]	Average Growth Rate µ [day^{-1}] and Inhibition Ir [%]					
		0-24 hours		0-48 hours		0-72 hours	
		µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]
Control	---	1.921	0.0	1.935	0.0	1.855	0.0
14	9.0	1.969	-2.5	1.924	0.5	1.855	0.0
28	19	1.917	0.2	1.902	1.7	1.840	0.8
56	37	1.888	1.7	1.850*	4.4	1.812*	2.3
113	74	1.825	5.0	1.759*	9.1	1.739*	6.2
225	149	1.544*	19.6	1.284*	33.6	1.129*	39.1
450	311	1.264*	34.2	0.688*	64.4	0.470*	74.6

** Mean value statistically significantly lower than in the control (according to a Williams' t-test, one-sided smaller, α = 0.05).*

Table B.2.9.2.2-72: Effect on Yield (Y) and percent inhibition for *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Mean Measured Test Item Concentration [µg/L]	Yield Y (× 10^4) and Inhibition Iy [%]					
		0-24 hours		0-48 hours		0-72 hours	
		Y	I_y [%]	Y	I_y [%]	Y	I_y [%]
Control	---	4.98	0.0	39.69	0.0	219.99	0.0
14	9.0	5.24	-5.2	38.84	2.1	219.99	0.0
28	19	4.92	1.1	37.09*	6.5	210.38	4.4
56	37	4.75	4.5	33.33*	16.0	192.96*	12.3
113	74	4.41	11.5	27.76*	30.1	155.24*	29.4
225	149	3.12*	37.4	10.20*	74.3	24.19*	89.0
450	311	2.15*	56.8	2.51*	93.7	2.62*	98.8

* Mean value statistically significantly lower than in the control (according to a Williams' t-test, one-sided smaller, α = 0.05).

Assessment and conclusions:

The test item Methyl isothiocyanate (MITC) had acute toxic effects to *Pseudokirchneriella subcapitata* in a 72 hour static test.
According to the growth rate, the 72 hour EC_{50} was calculated to be 189 µg/L; the 95 %-confidence limits were 187 and 191 µg/L. With regard to the yield, the 72 hour EC_{50} was calculated to be 91 µg/L; the 95 %-confidence limits were 88 and 93 µg/L. Concentrations are based on mean measured concentrations.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 189 µg MITC/L (mean measured) (95 % confidence limits: 187 – 191 µg/L)

E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 91 µg MITC/L (mean measured) (95 % confidence limits: 88 – 93 µg/L)
Analytical method:
Method validation acceptable according to SANCO/3029/99 rev. 4.
Assessment and conclusion by Lainco:
The study is acceptable.
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 189 µg MITC/L (mean measured) (95 % confidence limits: 187 – 191 µg/L)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 104 µg MITC/L (mean measured) (95 % confidence limits: 103 – 106 µg/L)
E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 76 µg MITC/L (mean measured) (95 % confidence limits: 75 – 78 µg/L)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: factor of 261)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 10 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 7 % (measured: 1.0 %)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/68 (Taminco) and KCA 4.1.2/25 (Lainco), for further details).
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 189 µg MITC/L (mean measured) (95 % confidence limits: 187 – 191 µg/L)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 104 µg MITC/L (mean measured) (95 % confidence limits: 103 – 106 µg/L)
E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 76 µg MITC/L (mean measured) (95 % confidence limits: 75 – 78 µg/L)
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 91 µg MITC/L (mean measured) (95 % confidence limits: 88 – 93 µg/L)
E_yC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 62 µg MITC/L (mean measured) (95 % confidence limits: 60 – 64 µg/L)
E_yC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 51 µg MITC/L (mean measured) (95 % confidence limits: 48 – 53 µg/L)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.1/10.

Data point:	KCA 8.2.6.1/10
Report author:	[redacted]
Report year:	2020d

Report title:	Methyl isothiocyanate (MITC) - Effect on *Pseudokirchneriella subcapitata* in a 72-Hour Algal Growth Inhibition Test – Statistical Re-analysis.
Report No.:	CEA.2143
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report from Innovative Environmental Services (IES) Ltd., Study code 20180073 ([redacted], 2018; KCA 8.2.6.1/09), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the freshwater algae *Pseudokirchneriella subcapitata* did not provide estimates of the EC_{10}, EC_{20} or EC_{50} for the biomass integral over the total growth curve (area under the growth curve). Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal measured concentrations of MITC at 14, 28, 56, 113, 225 and 450 µg/L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 70 % to 72 % of the nominal values from test initiation and from 52 % to 66 % in samples on day 3 of the test. In line with the original study, the geometric mean measured test concentrations were used for this re-analysis, the concentrations were 9, 19, 37, 74, 149 and 311 µg/L.

The following parameters were analysed statistically:

- Biomass (b)

Statistical analyses of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-73: Summary of effects on *Pseudokirchneriella subcapitata*, exposed to MITC for 72 hours under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours			48 hours			72 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg/L]	55.0	91.9	45.6	58.3	93.3	112.2	45.6	58.3	93.3
lower 95 %-cl	30.9	63.5	43.6	56.4	91.4	107.6	43.6	56.4	91.4
upper 95 %-cl	76.0	115.6	47.5	60.1	95.3	117.0	47.5	60.1	95.3

cl: confidence limit

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 189 µg MITC/L (mean measured) (95 % confidence limits: 187 – 191 µg/L)
E_yC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 91 µg MITC/L (mean measured) (95 % confidence limits: 88 – 93 µg/L)
Endpoints from re-analysis:
Statistical analyses of the available data for biomass revealed that the following E_bC_{50}, E_bC_{20} and E_bC_{50} values were reliably calculated:
E_bC_{10} (*Pseudokircheneriella subcapitata*, 72 h) = 45.6 µg MITC/L (mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h) = 58.3 µg MITC/L (mean measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h) = 93.3 µg MITC/L (mean measured)
Assessment and conclusion by Lainco:
The study is acceptable.
E_rC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 189 µg MITC/L (mean measured) (95 % confidence limits: 187 – 191 µg/L)
E_rC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 104 µg MITC/L (mean measured) (95 % confidence limits: 103 – 106 µg/L)
E_rC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 76 µg MITC/L (mean measured) (95 % confidence limits: 75 – 78 µg/L)

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analyses of the available data for biomass revealed that the following E_bC_{50}, E_bC_{20} and E_bC_{50} values were reliably calculated:
E_bC_{10} (*Pseudokircheneriella subcapitata*, 72 h, static) = 45.6 µg MITC/L (mean measured)
E_bC_{20} (*Pseudokircheneriella subcapitata*, 72 h, static) = 58.3 µg MITC/L (mean measured)
E_bC_{50} (*Pseudokircheneriella subcapitata*, 72 h, static) = 93.3 µg MITC/L (mean measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.2/01
Report author:	[redacted]
Report year:	2002
Report title:	Effect of Methyl isothiocyanate (MITC) on the Growth of the Blue-green Alga *Anabaena flos-aquae*.
Report No.:	106691
Document No.:	2002/1006170
Guidelines followed in study:	ASTM E 1218-90, considering OECD 201 (1984) and OPPTS 850.1000 (1996)
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities

Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

The freshwater blue-green alga *Anabaena flos-aquae* was exposed for 72 hours to Methyl isothiocyanate (MITC) nominal concentrations of 1, 2, 3.8, 7.4, 14, 27 and 50 mg/L (initial measured concentrations: 0.9, 1.5, 2.7, 5.0, 8.9, 17.4 and 28.9 mg/L), each with 5 replicates, and a dilution water control. The control was run with 10 replicates.
Measured MITC concentration at test initiation ranged from 57.7 % to 93.5 % of nominal. After 72 hours measured concentrations declined to 3.5 % to 5.7 % of nominal.
Derived 72 hour E_yC_{50} and E_yC_{10} based on initial measured concentrations were 2.12 mg MITC/L (95 % confidence limits: 2.04 – 2.20 mg MITC/L) and 0.65 mg MITC/L (95 % confidence limits: 0.61 – 0.70 mg MITC/L), respectively. Growth rate based E_rC_{50} value was 3.72 mg MITC/L (95 % confidence limits: 3.60 – 3.85 mg MITC/L) and E_rC_{10} was 1.23 mg MITC/L (95 % confidence limits: 1.16 – 1.30 mg MITC/L).

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 408208/1, chemical purity: 99.6 %
Test species:	Blue-green algae (*Anabaena flos-aquae*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 1.0, 2.0, 3.8, 7.4, 14, 27 and 50 mg MITC/L
Dilution medium:	AAP algal medium
Initial cell density, number of replicates:	3 x 10^4 cells/mL, 10 replicates for the control and 5 replicates per treatment group
Time of exposure:	72 hours In-life dates: May 14th to 17th 2002
Test conditions:	temperature: 24 ± 1 °C pH: 7.5 at start, 7.44 – 7.63 at end photoperiod: continuous illumination light intensity: 2300 lux
Test procedure:	The freshwater blue-green alga *Anabaena flos-aquae* was exposed for 72 hours to nominal concentrations of Methyl isothiocyanate (MITC) 1, 2, 3.8, 7.4, 14, 27 and 50 mg/L (initial measured concentrations: 0.9, 1.5, 2.7, 5.0, 8.9, 17.4 and 28.9 mg/L), each with 5 replicates, and a dilution water control. The control was run with 10 replicates. A stock solution was prepared by adding 50 mg of the test substance to 1000 mL nutrient solution and stirring for about 20 min. The stock solution was diluted with algal nutrient solution to reach the desired concentrations. Flasks were inoculated with algae from a pre-culture to get an initial cell concentration of about 3 x 10^4 cells/mL. The culture flasks were placed in a temperature controlled incubator until sampling. During the experiment the algae were kept in suspension by constant shaking at about 170 rpm.
Test item analysis:	At the beginning and at the end of the test samples were taken for verification of test substance concentrations. Because of rapid

hydrolytic degradation, the samples for the analytical measurements were acidified with 0.1 % (v/v) hydrochloric acid (10 M). At the last sampling interval, the pH of all individual samples (controls as well as treated samples) was measured. The test substance is quantified by reversed phase HPLC. Quantitation is achieved by UV detection of MITC at 248 nm and external calibration using the test substance MITC as reference substance.

Observations: The cell concentration in each flask was determined 24, 48 and 72 hours after starting the experiment with a spectrophotometer (445 nm, 5 cm glass cuvettes). Algal medium was used as blank. To obtain the actual number of cells/mL a linear correlation (calibration curve) was calculated from the cell numbers (counted under a microscope) versus extinction values. pH was measured after 72 hours.

Statistical evaluation: At each sampling interval one aliquot of each of the replicates of test and control flasks was collected for evaluation of algal densities. Subsequently the mean values per treatment were calculated and growth curves plotted. The percent inhibition of the cell growth at each test concentration is calculated from the difference between the area under the control growth curve and the area under the growth curve at each test concentration. The mathematical determination of the EC_x was done by probit analysis. The probit analysis was done with permille inhibition values (to get integer figures) and accordingly an assumed standard number of individuals of 1000. The calculations were conducted with a PC and the commercial software "TOXSTAT 3.5" (WEST, Inc. and Dave Gulley, University of Wyomimg, USA).

Findings:

Analytical results: Measured MITC concentration at test initiation ranged from 57.7 to 93.5 % of nominal. After 72 hours measured concentrations declined to 3.5 to 5.7 % of nominal. The low recovery at test termination is due to the hydrolytic instability of MITC. Derived effect concentrations are based on initial measured MITC concentrations.

Detailed results on measured MITC concentrations are presented in the table below.

Inhibition of biomass and growth: No morphological effects on the algae could be observed at concentrations up to 5 mg/L (initial). At 8.9 mg MITC/L a few, at 27 mg MITC/L about a fourth and at the highest test concentration all cells appeared deformed.

After 72 hours of exposure, inhibition of yield relative to the negative control in the 0.9, 1.5, 2.7, 5, 8.9, 17.4 and 28.9 mg MITC/L (initial measured) treatment groups was 0 %, 489 %, 679 %, 877 %, 947 %, 963 % and 976 %, respectively. Inhibition of growth rate relative to the negative control in the 0.9, 1.5, 2.7, 5, 8.9, 17.4 and 28.9 mg MITC/L treatment groups was 0 %, 223 %, 317 %, 706 %, 811 %, 941 % and 1000%, respectively.

Detailed results on biomass and cell growth are presented in the tables below.

Table B.2.9.2.2-74: Measured concentrations of MITC in exposure algal medium at test initiation (0 hours) and at test termination (72 hours) of the Algal Growth Inhibition Test with *Anabaena flos-aquae*

Nominal concentration (mg MITC/L)	Mean measured concentrations (mean of two samples) (mg MITC./L)					% of nominal concentration
	0 hours	% of nominal	72 hours	% of initial measured	Mean	
1	0.935	93.5	0.051	5.45	0.493	21.8
2	1.47	73.7	0.11	7.48	0.79	20.1
3.8	2.66	69.9	0.20	7.52	1.43	19.2
7.4	5.02	67.8	0.26	5.18	2.64	24.3
14	8.95	63.9	0.62	6.93	4.79	16.8
27	17.37	64.3	1.41	8.12	9.39	18.3
50	28.85	57.7	2.32	8.04	15.6	16.4

Table B.2.9.2.2-75: Mean cell numbers and percent inhibition in biomass of *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Day 0 Measured test concentrations (mg MITC./L)	24 hours	48 hours	72 hours	% Inhibition in biomass
	Mean cell number (cells/mL)	Mean cell number (cells/mL)	Mean cell number (cells/mL)	
control	105300	445000	1934000	-
0.9	108300	455500	1990000	-2.9
1.5	82820	345700	766400	48.9
2.7	68010	205800	527500	67.9
5.0	51680	149700	102200	87.7
8.9	53720	64950	65970	94.7
17.4	59330	49630	38400	96.3
28.9	46570	49630	26150	97.6

Table B.2.9.2.2-76: Mean growth rate per day and percent inhibition of *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Day 0 measured test concentrations (mg MITC/L)	24 hours	48 hours	72 hours	Average 0-72 h	% Inhibition
	Mean growth rate (per day)	Mean growth rate (per day)	Mean growth rate (per day)		
control	1.255	1.442	1.469	1.388	-
0.9	1.282	1.437	1.474	1.398	-0.7
1.5	1.015	1.429	0.791	1.078	22.3
2.7	0.818	1.107	0.920	0.948	31.7
5.0	0.544	1.058	-0.378	0.408	70.6
8.9	0.582	0.190	0.016	0.263	81.1
17.4	0.678	-0.175	-0.258	0.082	94.1
28.9	0.370	0.133	-1.205	-0.234	100.0

Assessment and conclusions:

The freshwater blue-green alga *Anabaena flos-aquae* was exposed for 72 hours to nominal concentrations of Methyl isothiocyanate (MITC) of 1, 2, 3.8, 7.4, 14, 27 and 50 mg/L.

The following biological results are based on initial measured concentrations:

E_rC_{50} (0 – 72 h) = 3.72 mg MITC/L (95 % confidence limits: 3.60 – 3.85 mg MITC/L)

E_rC_{10} (0 – 72 h) = 1.23 mg MITC/L (95 % confidence limits: 1.16 – 1.30 mg MITC/L)
E_yC_{50} (0 – 72 h) = 2.12 mg MITC/L (95 % confidence limits: 2.04 – 2.20 mg MITC/L)
E_yC_{10} (0 – 72 h) = 0.65 mg MITC/L (95 % confidence limits: 0.61 – 0.70 mg MITC/L)

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 3.72 mg MITC/L (initial measured)
E_rC_{10} (*Anabaena flos-aquae*, 72 h) = 1.23 mg MITC/L (initial measured)
E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 2.12 mg MITC/L (initial measured)
E_yC_{10} (*Anabaena flos-aquae*, 72 h) = 0.65 mg MITC/L (initial measured)
Analytical method:
Method validation not fit for purpose. This study is submitted for information only and is not relied upon for risk assessment.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:
- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 10 %

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
No further information on the analytical method used.
E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 3.72 mg MITC/L (initial measured)
E_rC_{10} (*Anabaena flos-aquae*, 72 h, static) = 1.23 mg MITC/L (initial measured)
E_yC_{50} (*Anabaena flos-aquae*, 72 h, static) = 2.12 mg MITC/L (initial measured)
E_yC_{10} (*Anabaena flos-aquae*, 72 h, static) = 0.65 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.2/02.

Data point:	KCA 8.2.6.2/02
Report author:	[redacted]
Report year:	2019b
Report title:	Effect of Methyl isothiocyanate (MITC) on the Growth of the Blue-green Alga *Anabaena flos-aquae* – Statistical Re-analysis.
Report No.:	CEA.2035
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted:

	OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from BASF Aktiengesellschaft. Study code 106691 ([redacted] 2002; KCA 8.2.6.2/01) for the toxicity of Methyl isothiocyanate (MITC) on the growth of the blue-green alga *Anabaena flos-aquae* did not provide estimates of the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.
The test used nominal concentrations of Methyl isothiocyanate (MITC) at 1, 2, 3.8, 7.4, 14, 27 and 50 mg/L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 57.7 % to 93.5 % of the nominal values from test initiation and 3.5 % to 5.7 % in samples at the end of the test. In line with the original study, the initial measured test concentrations were used for this analysis, the concentrations were 0.9, 1.5, 2.7, 5.0, 8.9, 17.4 and 28.9 mg/L.

The following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)
- Biomass (b)

Statistical analyses of the available data for **yield** revealed that the following E_yC_{10}, E_yC_{20} and E_yC_{50} values were reliably calculated:

Table B.2.9.2.2-77: Summary of effects on *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions, statistical re-analysis for yield, based on mean measured values

Parameter	24 hours		48 hours			72 hours		
	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg a.i./L]	0.815	4.366	0.943	1.358	2.728	0.801	1.009	1.569
lower 95 %-cl	0.378	3.199	0.798	1.201	2.538	0.631	0.849	1.434
upper 95 %-cl	1.296	5.901	1.078	1.503	2.933	0.934	1.134	1.722

cl: confidence limits

Statistical analyses of the available data for **average specific growth rate** revealed that the following E_rC_{10}, E_rC_{20} and E_rC_{50} values were reliably calculated:

Table B.2.9.2.2-78: Summary of effects on *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions, statistical re-analysis for growth rate, based on mean measured values

Parameter	24 hours		48 hours			72 hours		
	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}

Value [mg a.i./L]	1.258	10.160	1.305	2.199	5.970	1.143	1.696	3.607
lower 95 %-cl	0.540	7.262	1.062	1.890	5.462	0.954	1.484	3.334
upper 95 %-cl	2.056	15.630	1.547	2.500	6.529	1.322	1.894	3.903

cl: confidence limits

Statistical analyses of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-79: Summary of effects on *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions, statistical re-analysis for biomass, based on mean measured values

Parameter	24 hours		48 hours			72 hours		
	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [mg/L]	0.815	4.366	0.822	1.269	2.910	0.793	1.068	1.886
lower 95 %-cl	0.598	3.787	0.771	1.210	2.827	0.764	1.038	1.853
upper 95 %-cl	1.041	5.023	0.873	1.327	2.995	0.822	1.097	1.919

cl: confidence limit

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 3.72 mg MITC/L (initial measured)
E_rC_{10} (*Anabaena flos-aquae*, 72 h) = 1.23 mg MITC/L (initial measured)
E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 2.12 mg MITC/L (initial measured)
E_yC_{10} (*Anabaena flos-aquae*, 72 h) = 0.65 mg MITC/L (initial measured)
Endpoints from re-analysis:
E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 3.607 mg MITC/L (initial measured)
E_rC_{10} (*Anabaena flos-aquae*, 72 h) = 1.143 mg MITC/L (initial measured)
E_bC_{50} (*Anabaena flos-aquae*, 72 h) = 1.886 mg MITC/L (initial measured)
E_bC_{10} (*Anabaena flos-aquae*, 72 h) = 0.793 mg MITC/L (initial measured)
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 3.607 mg MITC/L (initial measured)
E_rC_{10} (*Anabaena flos-aquae*, 72 h, static) = 1.143 mg MITC/L (initial measured)
E_bC_{50} (*Anabaena flos-aquae*, 72 h, static) = 1.886 mg MITC/L (initial measured)
E_bC_{10} (*Anabaena flos-aquae*, 72 h, static) = 0.793 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.2/03
Report author:	[redacted]
Report year:	2012a
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Freshwater Alga (*Anabaena flos-aquae*).

Report No.:	703A-104
Document No.:	-
Guidelines followed in study:	OECD 201 (2006) EU Directive 92/69/EEC, Method C.3 US EPA OPPTS Number 850.5400 ISO 14442 Standard
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

In a static acute toxicity test, the freshwater blue-green alga *Anabaena flos-aquae* was exposed for 96 hours to 6 nominal concentrations ranging between 10 and 1000 µg Methylisothiocyanat (MITC)/L. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL. Measured concentrations declined by day 4, and ranged from 52 % to 85 % of nominal. The results of the study are based on day 0 measured concentrations of 11, 24, 56, 129, 317 and 749 µg a.s./L, representing 107 %, 91 %, 87 %, 81 %, 79 % and 75 % of the target nominal test concentrations.
Effects were evaluated based on yield and growth rate. The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 433 (355 to 527) µg a.s./L and 319 (< 11 to >749) µg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 341 (253 to 459) µg a.s./L and 200 (123 to 326) µg a.s./L. The 72 and 96 hour no observed adverse effect concentrations (NOAEC), based on effects on yield and growth rate were 317 and 129 µg a.s./L, respectively.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 99.7 %
Test species:	Blue-green algae (*Anabaena flos-aquae*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 10, 26, 64, 160, 400 and 1000 µg MITC/L
Dilution medium:	AAP algal medium
Initial cell density, number of replicates:	5000 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	96 hours In-life dates: September 22nd to 26th 2011
Test conditions:	temperature: 23.1 – 23.8 °C pH: 7.7 – 7.8 at start, 8.1 – 9.4 at end photoperiod: continuous illumination light intensity: 1970 - 2230 lux
Test procedure:	The freshwater blue-green alga *Anabaena flos-aquae* was exposed for 96 hours to nominal concentrations of 0, 10, 26, 64, 160, 400 and 1000 µg a.s./L. Three replicate test chambers were maintained

	in each treatment group and six replicates were maintained in the control group. A single abiotic replicate was maintained at the highest test concentration to assess stability of the test substance under test conditions. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL.
Test item analysis:	Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group prior to distribution into the test chambers. At exposure termination, samples were collected from the pooled replicates from each treatment and control group. Samples were collected into glass scintillation vials and acidified with two drops of 10 % phosphoric acid prior to analysis The analytical method consisted of diluting the samples in acidified freshwater AAP NaHCO3 algal medium, extracting the samples with diethyl ether and analyzing by gas chromatography with mass selective detection. The samples were analysed by GC-MS.
Observations:	Test medium samples were collected from each replicate of the treatment and control groups for the determination of algal cell densities. Samples were collected at approximately 24 hour intervals during the 96 hour exposure using a syringe and were held for a maximum of three days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts were performed using a hemacytometer and a microscope. Samples were syringed prior to counting to break up and disperse any filaments of Anabaena that may have formed during the study period. Each sample was diluted using an electrolyte solution (Isoton®), as needed, to maintain counting accuracy. A small amount of each sample was loaded onto a hemacytometer and the total number of *Anabaena* cells was counted in 10 grids to calculate the cell density of the sample. Samples of test solution were collected from each of the replicates per treatment and control group at the end of the test. These samples were pooled within their respective treatments, and subsamples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregation or flocculation of cells, and adherence of the cells to the test chamber.
Statistical evaluation:	The calculation of cell densities, yield, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using “The SAS System for Windows, Version 8.2”. Growth rate was calculated for each replicate of the control and treatment groups. Yield was calculated for each replicate of the control and treatment groups as the final biomass (cell density) in the exposure period minus the initial biomass (cell density). Inhibition values were calculated for each treatment group as the percent reduction in yield and growth rate relative to the negative control replicates for each 24 hour interval. The E_yC_{50} and E_rC_{50} values and their 95 % confidence intervals were calculated, when possible, using non-

linear regression with replicate data (yield or growth rate) and day 0 measured test concentrations. Non-linear regression analyses were conducted based on the measured MITC concentrations in samples collected at test initiation (hour 0). The 72 and 96 hour yield and growth rate data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro-Wilk's and Levene's tests, respectively. The 96 hour growth rate data failed the Shapiro-Wilk's test for normal distribution. Log transformation of the data failed to resolve the problem. However, Dunnett's test is considered robust with respect to small departures from normality, and therefore the treatment groups were compared to the negative control using Dunnett's one-tailed t-test ($\alpha = 0.05$). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 and 96 hours.

Findings:

Analytical results:

Results of analyses to measure concentrations of MITC in test solution samples collected during the study. Nominal test concentrations were 10, 26, 64, 160, 400 and 1000 µg a.s./L. The test solutions appeared clear and colorless at the time of preparation, and there was no evidence of surface slicks or precipitation at exposure termination. Measured concentrations on day 0 ranged from 75 to 107 % of nominal. Measured concentrations declined by day 4, and ranged from 52 to 85 % of nominal. The results of the study are based on the day 0 measured concentrations of 11, 24, 56, 129, 317 and 749 µg a.s./L, representing 107, 91, 87, 81, 79 and 75 % of the target nominal test concentrations. The measured concentration in the abiotic control was 75 % of nominal at test initiation and 74 % of nominal at test termination.

Inhibition of biomass and growth:

After 72 hours of exposure, inhibition of yield in the 11, 24, 56, 129, 317 and 749 µg a.s./L treatment groups was 3 %, -50 %, -4 %, 22 %, 38 % and 98 %, respectively, relative to the negative control. Inhibition of growth rate in the 11, 24, 56, 129, 317 and 749 µg a.s./L treatment groups was 3 %, -13 %, 6 %, 8 %, 18 % and 95 %, respectively, relative to the negative control. Dunnett's test indicated that there was a significant reduction ($p \leq 0.05$) in yield and growth rate in the 749 µg a.s./L treatment group. Consequently, the 72 hour NOAEC for yield and growth rate was 317 µg a.s./L.

After 96 hours of exposure, inhibition of yield in the 11, 24, 56, 129, 317 and 749 µg a.s./L treatment groups was 30 %, 27 %, 21 %, 25 %, 91 % and 100 %, respectively, relative to the negative control. Inhibition of growth rate in the 11, 24, 56, 129, 317 and 749 µg a.s./L treatment groups was 2 %, -1 %, -3 %, 2 %, 48 % and 100 %, respectively, relative to the negative control. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 317 and 749

µg a.s./L treatment groups. Consequently, the 96 hour NOAEC for yield and growth rate was 129 µg a.s./L.

After 96 hours of exposure, there were flocculations or aggregations of cells in all of the experimental groups. *Anabaena flos-aquae* forms long chains of cells, aggregations of cells are a characteristic of this particular algal species. Adherence of cells to the test chambers was not observed in the negative control or any of the treatment groups. Cells in the treatment groups appeared normal when compared to cells present in the negative control. No morphological deformities were observed in any of the treatment groups.

Table B.2.9.2.2-80: Mean yield and percent inhibition of *Anabaena flos-aquae*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentrations (µg a.s./L)	24 hours[1]		48 hours[1]		72 hours[1]		96 hours[1]	
	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]
negative control	7167	--	48333	--	90667	--	442500	--
11	12333	-72	53000	-10	88333	3	310667	30
24	16000	-123	40667	16	135667	-50	323667	27
56	17667	-147	72000	-49	94000	-4	350667	21
129	1000	86	12000	75	70667	22	331000	25
317	0	100	19333	60	56000	38	38333*	91
749	1667	77	2333	95	1000*	99	0*	100

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.

[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.

** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Table B.2.9.2.2-81: Mean growth rate (per hour) and percent inhibition of *Anabaena flos-aquae*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentrations (µg a.s./L)	24 hours[1]		48 hours[1]		72 hours[1]		96 hours[1]	
	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]	Mean growth rate (hours)	% Inhibition[2]
negative control	0.0305	--	0.0481	--	0.0407	--	0.0432	--
11	0.0419	-37	0.0495	-3	0.0396	3	0.0425	2
24	0.0574	-88	0.0452	6	0.0462	-13	0.0434	-1
56	0.0511	-68	0.0527	-9	0.0384	6	0.0444	-3
129	0.0065	79	0.0220	54	0.0376	8	0.0425	2
317	0.0000	100	0.0324	33	0.0332	18	0.0224*	48
749	0.0096	68	0.0073	85	0.0022*	95	0.0000*	100

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.

[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.

** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Assessment and conclusions:

The freshwater blue-green (cyanobacteria) alga, *Anabena flos-aquae*, was exposed to a geometric series of six concentrations of MITC ranging from 11 to 749 µg a.s./L, based on measured concentrations of MITC at test initiation (day 0). Effects were evaluated based on yield and growth rate. The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 433 (355 to 527) µg a.s./L and 319 (< 11 to >749) µg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 341 (253 to 459) µg a.s./L and 200 (123 to 326) µg a.s./L. The 72 and 96 hour no observed adverse effect concentrations (NOAEC), based on effects on yield and growth rate were 317 and 129 µg a.s./L, respectively.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 0.433 mg MITC/L (initial measured)

E_rC_{50} (*Anabaena flos-aquae*, 96 h) = 0.319 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 0.341 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 96 h) = 0.200 mg MITC/L (initial measured)

Analytical method:

Method validation not fully acceptable according to the guidance SANCO/3029/99 rev. 4 on analytical validation, but is accepted as fit for purpose.

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is considered to be in line with the current guidance.

The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: 18.1)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 %
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 10 %

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.

Therefore, this study is still considered acceptable.

The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered “fit for purpose” (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/59, for further details).

The study is relied upon, though the endpoint is not critical.

E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 0.433 mg MITC/L (initial measured)

E_rC_{50} (*Anabaena flos-aquae*, 96 h, static) = 0.319 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 72 h, static) = 0.431 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 96 h, static) = 0.200 mg MITC/L (initial measured)

This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.2/04.

Data point:	KCA 8.2.6.2/04
Report author:	[redacted]
Report year:	2020
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Freshwater Alga (*Anabaena flos-aquae*) – Statistical Re-analysis.
Report No.:	CEA.2137
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from Wildlife International, Ltd., Study code 703A-104 ([redacted] 2012; KCA 8.2.6.2/03), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the blue-green alga *Anabaena flos-aquae* did not provide values for the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. Additionally, no statistical analysis for biomass was provided. Consequently, the data has been re-analysed in this study in an attempt to provide these values.

The test used nominal concentrations of MITC at 10, 26, 64, 160, 400 and 1000 µg a.s./L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 75 % to 107 % of the nominal values at test initiation and reduced to 52 % to 85 % of nominals by the end of the study (day 4). In line with the original study, the initial mean measured test concentrations were used for these re-analyses, the concentrations were 11, 24, 56, 129, 317 and 749 µg a.s./L.

The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (µ)
- Biomass (b)

Statistical re-analysis of the available data for **yield after 24 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was significant, p(F) = < 0.001, and the slope function was 1.347. However, the EC_{10} and EC_{20} values were not considered to be reliable as no confidence levels could be calculated.

Statistical re-analysis of the available data for **yield after 48 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response at 48 hours was not significant, p(F) = 0.1, and the slope function was 3.103. As a result, no EC_{10} or EC_{20} values could be calculated.

Statistical re-analysis of the available data for **yield after 72 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was significant, p(F) = 0.033, and the slope function was 2.012. However, the EC_{10} and EC_{20} values are not considered reliable as the 95 % confidence limits could not be determined.

Statistical re-analysis of the available data for **yield after 96 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response at 96 hours was not significant, p(F) = 0.105, and the slope function was 4.281. As a result, no EC_{10} and EC_{20} values could be calculated.

Statistical re-analysis of the available data for **average specific growth rate after 24 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was not significant, p(F) = >0.05, and the slope function was 0.711. As a result, no EC_{10} and EC_{20} values could be calculated.

Statistical re-analysis of the available data for **average specific growth rate after 48 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was not significant, p(F) = 0.117, and the slope function was 3.390. As a result, no EC_{10} and EC_{20} values could be calculated.

Statistical re-analysis of the available data for **average specific growth rate after 72 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was not significant, p(F) = 0.708, and the slope function was 1.221. As a result, no EC_{10} and EC_{20} values could be calculated.

Statistical re-analysis of the available data for **average specific growth rate after 96 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was significant, p(F) = <0.001, and the slope function was 1.414. However, the EC_{10} and EC_{20} values are not considered reliable as the 95 % confidence levels could not be determined.

Statistical re-analysis of the available data for **biomass after 24 hours** revealed that no E_bC_{10}, E_bC_{20} or E_bC_{50} could be reliably calculated. The relationship between dose and response was not significant, p(F) = 0.09, and the slope function was 19.721. As a result, no EC_{10}, EC_{20} or EC_{50} values could be calculated.

Statistical re-analysis of the available data for **biomass after 48 hours** revealed that no E_bC_{10}, E_bC_{20} or E_bC_{50} could be reliably calculated. The relationship between dose and response was found to be significant, p(F) = <0.001, and the slope function was 1.522. However the EC_{10} and EC_{20} values were not considered reliable as the 95 % confidence limits could not be determined and the EC_{50} value was not reliable due to the confidence limits spanning more than two test concentrations.

Statistical re-analysis of the available data for **biomass after 72 hours** revealed that no E_bC_{10}, E_bC_{20} or E_bC_{50} could be reliably calculated. The relationship between dose and response was significant, p(F) = <0.001, and the slope function was 3.309. However the EC_{20} and EC_{50} values were not considered to be reliable due to wide confidence limits. The EC_{10} value was not considered to be reliable as no 95 % confidence limits could be determined.

Statistical analyses of the available data for **biomass after 96 hours** revealed that the following EC_{10}, EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.2-82: Summary of effects on *Anabaena flos-aquae*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	96 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg a.i./L]	61.7	89.0	179.2
lower 95 %-cl	16.7	35.5	122.9
upper 95 %-cl	97.3	128.3	263.8

cl: confidence limit

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 0.433 mg MITC/L (initial measured)

E_rC_{50} (*Anabaena flos-aquae*, 96 h) = 0.319 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 0.341 mg MITC/L (initial measured)

E_yC_{50} (*Anabaena flos-aquae*, 96 h) = 0.200 mg MITC/L (initial measured)

Endpoints from re-analysis:

Statistical re-analysis of the available data for both yield and growth rate revealed that no reliable EC_{10} or EC_{20} values could be calculated.

Statistical re-analysis of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be calculated:

E_bC_{10} (*Anabaena flos-aquae*, 96 h) = 61.7 µg MITC/L (initial measured)

E_bC_{20} (*Anabaena flos-aquae*, 96 h) = 89.0 µg MITC/L (initial measured)

E_bC_{50} (*Anabaena flos-aquae*, 96 h) = 179.2 µg MITC/L (initial measured

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is still considered acceptable.

Endpoints from re-analysis:

Statistical re-analysis of the available data for both yield and growth rate revealed that no reliable EC_{10} or EC_{20} values could be calculated.

Statistical re-analysis of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be calculated:

E_bC_{10} (*Anabaena flos-aquae*, 96 h, static) = 61.7 µg MITC/L (initial measured)

E_bC_{20} (*Anabaena flos-aquae*, 96 h, static) = 89.0 µg MITC/L (initial measured)

E_bC_{50} (*Anabaena flos-aquae*, 96 h, static) = 179.2 µg MITC/L (initial measured

This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.2/05
Report author:	[redacted]
Report year:	2012b
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Freshwater Diatom (*Navicula pelliculosa*).
Report No.:	703A-105A
Document No.:	-

Guidelines followed in study:	The protocol was based on procedures outlined in: OECD 201 (2006) EU Directive 92/69/EEC, Method C.3 US EPA OPPTS Number 850.5400 ISO 14442 Standard
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): Aluminium seal closed bottles (recommendation: capping with air permeable stoppers) Initial algal biomass: 5000 cells/mL (recommendation: 10^4 cells/mL)
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

In a static acute toxicity test, the freshwater diatom *Navicula pelliculosa* was exposed for 96 hours to 5 nominal concentrations ranging between 63 and 1000 µg methyl isothiocyanate (MITC)/L. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL. Measured concentrations declined by day 4 and ranged from less than the limit of quantitation (< LOQ = 40.0 µg a.s./L) to 79 % of nominal. The results of the study are based on day 0 measured concentrations of 54, 104, 217, 431 and 837 µg a.s./L, representing 86 %, 83 %, 87 %, 86 %, 84 % and 84 % of the target nominal test concentrations.
The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 349 (301 to 406) µg a.s./L and 468 (390 to 562) µg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 181 (144 to 228) µg a.s./L and 314 (210 to 471) µg a.s./L. The 72 and 96 hour no-observed-adverse-effect-concentrations (NOAEC), based on effects on yield and growth rate were 104 and 217 µg a.s./L, respectively.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 99.7 %
Test species:	Freshwater diatom (*Navicula pelliculosa*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 63, 125, 250, 500 and 1000 µg MITC/L
Dilution medium:	AAP algal medium
Initial cell density, number of replicates:	5000 cells/mL, 8 replicates for the control and 4 replicates per treatment group
Time of exposure:	96 hours In-life dates: October 7th to 11th 2011
Test conditions:	temperature: 23.7 – 24.6 °C pH: 7.8 – 7.9 at start, 8.1 – 10.8 at end photoperiod: continuous illumination light intensity: 3890 - 4640 lux
Test procedure:	The freshwater diatom *Navicula pelliculosa* was exposed for 96 hours to nominal concentrations of 0, 63, 125, 250, 500 and 1000

	µg a.s./L. Four replicate test chambers were maintained in each treatment group and eight replicates were maintained in the control group. A single abiotic replicate was maintained at the highest test concentration to assess stability of the test substance under test conditions. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL.
Test item analysis:	Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group prior to distribution into the test chambers. At exposure termination, samples were collected from the pooled replicates from each treatment and control group. The abiotic sample was collected from replicate E of the 1000 µg a.s./L treatment group. Samples were collected into glass scintillation vials and acidified with two drops of 10 % phosphoric acid prior to analysis. The analytical method consisted of extracting the samples with diethyl ether and analyzing by gas chromatography with mass selective detection (GC-MS).
Observations:	Test medium samples were collected from each replicate of the treatment and control groups for the determination of algal cell densities. Samples were collected at approximately 24 hour intervals during the 96 hour exposure using a syringe and were held for a maximum of four days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts were performed using an electronic particle counter (Coulter Electronics, Inc.). Cell counts for samples collected during the test were conducted once instrument linearity was demonstrated (i.e., the R-squared value obtained through the regression analysis was at least 0.99935). A single aliquot of each sample collected during the test was diluted with an electrolyte solution (Isoton®). Three 0.5-mL volumes of the diluted sample were counted, and the resulting counts were averaged. The cell density of the sample was determined by adjusting the mean cell count (cells/mL) obtained using the particle counter, based upon the Y intercept and slope calculated through the regression analysis, and the dilution factor. Samples of test solution were collected from each replicate at the end of the test. These samples were pooled within their respective treatments, and sub-samples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregation or flocculation of cells, and adherence of the cells to the test chamber.
Statistical evaluation:	The calculation of cell densities, yield, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using “The SAS System for Windows, Version 8.2”. Growth rate was calculated for each replicate of the control and treatment groups. Yield was calculated for each replicate of the control and treatment groups as the final biomass (cell density) in the exposure period minus the initial biomass (cell density). Inhibition values were calculated for each treatment group as the percent reduction

in yield and growth rate relative to the negative control replicates for each 24 hour interval. The E_yC_{50} and E_rC_{50} values and their 95 % confidence intervals were calculated, when possible, using non-linear regression with replicate data (yield or growth rate) and day 0 measured test concentrations. Non-linear regression analyses were conducted based on the measured MITC concentrations in samples collected at test initiation (hour 0). The 72 and 96 hour yield and growth rate data were evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using Shapiro-Wilk's and Levene's tests, respectively. All data met assumptions of normal distribution and homogeneity of variance. The treatment groups were compared to the negative control using Dunnett's one-tailed t-test ($\alpha = 0.05$). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 and 96 hours.

Findings:

Analytical results:	Measured concentrations on day 0 ranged from 83 to 87 % of nominal. Measured concentrations declined by day 4, and ranged from less than the limit of quantitation (LOQ = 40.0 µg a.s./L) to 79 % of nominal. The results of the study are based on day 0 measured concentrations of 54, 104, 217, 431 and 837 µg a.s./L, representing 86, 83, 87, 86, 84 and 84 % of the target nominal test concentrations. The measured concentration of MITC in the abiotic control was 84 % of nominal at test initiation and 79 % of nominal at test termination.
Inhibition of biomass and growth:	Growth in the control replicates decreased from 72 to 96 hours. This decrease is probably due to the maximum cell density being achieved in the test system after 72 hours. Exponential growth, characterized by the linear section of the growth curve, occurred from 0 to 72 hours. After 72 hours of exposure, inhibition of yield in the 54, 104, 217, 431 and 837 µg a.s./L treatment groups was -6 %, -8 %, 66 %, 97 % and 99 %, respectively, relative to the negative control. Inhibition of growth rate in the 54, 104, 217, 431 and 837 µg a.s./L treatment groups was -2 %, -3 %, 21 %, 68 % and 79 %, respectively, relative to the negative control. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 217, 431 and 837 µg a.s./L treatment groups. Consequently, the 72 hour NOAEC for yield and growth rate was 104 µg a.s./L. After 96 hours of exposure, inhibition of yield in the 54, 104, 217, 431 and 837 µg a.s./L treatment groups was 5 %, -70 %, -53 %, 94 % and 97 %, respectively, relative to the negative control. Inhibition of growth rate in the 54, 104, 217, 431 and 837 µg a.s./L treatment groups was 0 %, -13 %, -11 %, 60 % and 72 %, respectively, relative to the negative control. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 431 and 837 µg a.s./L treatment groups. Consequently, the 96 hour NOAEC for yield and growth rate was 217 µg a.s./L. After 96 hours of exposure, there was aggregation or flocculation of cells in the negative control and all treatment groups. Adherence of cells to

the test chambers was noted in the negative control and the 54 and 104 µg a.s./L treatment groups. Cells in all treatment groups appeared normal when compared to cell in the negative control, during the microscopic examinations of the cells. Adherence of cells to the test chamber and aggregation of cells is commonly observed in studies conducted using a closed-system design and are not considered to be treatment related because these observations were noted in the negative control group.

Table B.2.9.2.2-83: Measured concentrations of MITC in test solution samples at test initiation (day 0) and at test termination (day 4) of the Algal Growth Inhibition Test with *Navicula pelliculosa*

Nominal concentration (µg a.s./L)	Measured concentration (µg a.s./L)[1]			Mean	% of nominal concentration
	Day 0	Day 4	% of day 0		
Control	< LOQ	< LOQ		-	-
63	54.3	< LOQ	0	37.2[4]	59
125	104	11.4[2]	10.9	57.7	46
250	217	133	61.3	175	70
500	431	358	83.1	395	79
1000	837	735	87.8	786	79
abiotic (1000)	837[3]	792	94.6	815	81

[1] The limit of quantitation (LOQ) was 40.0 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (40.0 µg a.s./L) and the dilution factor of the matrix blank samples (1.00). The limit of detection (LOD) was 5.85 µg a.s./L.

[2] Extrapolated value. Peak area was less than the lowest calibration standard. Measurement otherwise considered reliable.

[3] No separate analysis was conducted since the 1000 µg a.s./L and the abiotic control solution were allotted from the same batch of test solution at test initiation (day 0).

[4] Mean was calculated by using half of LOQ: 20.0 µg/L.

Table B.2.9.2.2-84: Mean yield and percent inhibition of *Navicula pelliculosa*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentrations (µg a.s./L)	24 hours[1]		48 hours[1]		72 hours[1]		96 hours[1]	
	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]	Mean yield (cells/mL)	% Inhibition[2]
negative control	42733	--	272313	--	667306	--	346814	--
54	44120	-3	193193	29	704417	-6	328395	5
104	29501	31	180715	34	721432	-8	589128	-70
217	20370	52	95090	65	224823*	66	531176	-53
431	11604	73	14091	95	19058*	97	22543*	94
837	5178	88	7620	97	8663*	99	10946*	97

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.

[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.

** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Table B.2.9.2.2-85: Mean growth rate (per hour and per day) and percent inhibition of *Navicula pelliculosa*, exposed to MITC for 96 hours under static test conditions

Day 0 Measured test concentra-tions (µg a.s./L)	0 - 24 hours[1]			0 - 48 hours[1]			0 - 72 hours[1]			0 - 96 hours[1]		
	Mean growth rate		I%	Mean growth rate		I%	Mean growth rate		I%	Mean growth rate		I%
	$hour^{-1}$	day^{-1}		$hour^{-1}$	day^{-1}		$hour^{-1}$	day^{-1}		$hour^{-1}$	day^{-1}	
negative control	0.0934	2.241	-	0.0826	1.9824	-	0.0674	1.618	-	0.0437	1.0488	-
54	0.0946	2.2704	-1	0.0761	1.8264	8	0.0687	1.649	-2	0.0436	1.0464	0
104	0.0802	1.9248	14	0.0752	1.8048	9	0.0691	1.658	-3	0.0492	1.1808	-13
217	0.0669	1.6056	28	0.0624	1.4976	25	0.0532*	1.277	21	0.0485	1.164	-11
431	0.0498	1.1952	47	0.0277	0.6648	66	0.0212*	0.509	68	0.0175*	0.42	60
837	0.0294	0.7056	68	0.0192	0.4608	77	0.0138*	0.331	79	0.0121*	0.2904	72

[1] Calculations were performed using the SAS System for Windows, Version 8.2. Manual calculations may differ slightly.
[2] Percent inhibition was calculated relative to the negative control replicates. Negative values indicate an increase relative to the negative control mean.
** Statistically significant reductions from the negative control mean using Dunnett's one-tailed t-test ($p \leq 0.05$).*

Assessment and conclusions:

Not all validity criteria were met in this study. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and 2-3) was 50.57 % exceeding the 35 % requirement. Failure to meet this criterion does not make this study invalid. This test employed a closed-bottle experimental design, which is slightly modified from the design of standard algal toxicity tests. In this particular design the total volume of test solution in each test chamber is reduced from 100 to 60 mL. This particular criterion is difficult to achieve due to cell densities in the control replicates reaching the maximum attainable cell density in the test system.

The freshwater diatom, *Navicula pelliculosa*, was exposed to a geometric series of five concentrations of MITC ranging from 54 to 837 µg a.s./L, based on measured concentrations on MITC at test initiation (day 0). Effects were evaluated based on yield and growth rate. The 72 and 96 hour E_rC_{50} values and corresponding 95 % confidence intervals, based on growth rate, were 349 (301 to 406) µg a.s./L and 468 (390 to 562) µg a.s./L, respectively. The 72 and 96 hour E_yC_{50} values and corresponding 95 % confidence intervals, based on yield, were 181 (144 to 228) µg a.s./L and 314 (210 to 471) µg a.s./L. The 72 and 96 hour no-observed-adverse-effect-concentrations (NOAEC), based on effects on yield and growth rate were 104 and 217 µg a.s./L, respectively.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Navicula pelliculosa*, 72 h) = 0.349 mg MITC/L (initial measured)
E_rC_{50} (*Navicula pelliculosa*, 96 h) = 0.468 mg MITC/L (initial measured)
E_yC_{50} (*Navicula pelliculosa*, 72 h) = 0.181 mg MITC/L (initial measured)
E_yC_{50} (*Navicula pelliculosa*, 96 h) = 0.314 mg MITC/L (initial measured)
Analytical method:
Method validation not fully acceptable according to the guidance SANCO/3029/99 rev. 4 on analytical validation, but is accepted as fit for purpose.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were not fully met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: 133)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 50.57 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 10 % (measured: 8.62 %)

As mentioned above in the study summary, the deviation of the mean coefficient of variation for section-by-section specific growth rates in the control cultures exceeded the validity criterion of 35 %. However, as explained in the study summary, this deviation can be explained and is related to the study design using closed bottles.
Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/60, for further details).
E_rC_{50} (*Navicula pelliculosa*, 72 h, static) = 0.349 mg MITC/L (initial measured)
E_rC_{50} (*Navicula pelliculosa*, 96 h, static) = 0.468 mg MITC/L (initial measured)
E_yC_{50} (*Navicula pelliculosa*, 72 h, static) = 0.181 mg MITC/L (initial measured)
E_yC_{50} (*Navicula pelliculosa*, 96 h, static) = 0.314 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.2/06.

Data point:	KCA 8.2.6.2/06
Report author:	[redacted]
Report year:	2020a
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Freshwater Diatom (*Navicula pelliculosa*) – Statistical Re-analysis.
Report No.:	CEA.2138
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report produced by Wildlife International Ltd., Study code 703A-105A ([redacted], 2012; KCA 8.2.6.2/05), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the freshwater diatom *Navicula pelliculosa* did not provide estimates of the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. In addition, the biomass integral over the total growth curve (area under the growth curve) was not calculated as part of the original study and estimates of the EC_x values were not provided. Consequently, the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal measured concentrations of MITC at 63, 125, 250, 500 and 1000 µg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 83.1 % to 86.7 % of the nominal values from test initiation and from < LOQ to 79.2 % in samples on day 4 of the test. In line with the original study, the initial mean measured test concentrations were used for this analysis, the concentrations were 54.3, 104, 217, 431 and 837 µg a.s./L.

The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (µ)
- Biomass (b)

Statistical analyses of the available data for **yield** revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table B.2.9.2.2-86: Summary of effects on *Navicula pelliculosa*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	24 hours		48 hours
	E_yC_{10}	E_yC_{20}	E_yC_{20}
Value [µg/L]	54.9	87.5	53.7
lower 95 %-cl	11.0	28.2	33.2
upper 95 %-cl	96.5	136.6	71.7

cl: confidence limits

Statistical re-analysis of the available data for **yield after 48 hours** revealed that no E_yC_{10} could be reliably calculated due to the EC value being more than 25 % below the lowest test concentration.

Statistical re-analysis of the available data for **yield after 72 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was non-significant, p(F) = 0.123, and the slope function was 1.270. As a result, the EC_{10} and EC_{20} values could not be determined.

Statistical re-analysis of the available data for **yield after 96 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was significant, p(F) = 0.007, and the slope function was 1.341. However, the EC_{10} and EC_{20} values are not considered reliable as the 95 % confidence levels could not be determined.

Statistical analyses of the available data for **average specific growth rate** revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Table B.2.9.2.2-87: Summary of effects on *Navicula pelliculosa*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for growth rate, based on initial measured values

Parameter	24 hours		48 hours		72 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [µg/L]	92.4	160.5	105.7	160.0	144.0	196.6
lower 95 %-cl	32.6	80.3	77.9	128.3	109.0	160.5
upper 95 %-cl	148.5	227.7	131.3	188.4	173.9	227.2

cl: confidence limit

Statistical re-analysis of the available data for **average specific growth rate after 96 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was not significant, p(F) = 0.062, and the slope function was 1.896. As a result, the EC_{10} and EC_{20} values could not be reliably determined.

Statistical analyses of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-88: Summary of effects on *Navicula pelliculosa*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	24 hours			48 hours		72 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg/L]	48.8	80.3	208.2	61.1	147.1	92.1	116.0	180.4
lower 95 %-cl	30.8	57.5	175.5	50.9	133.3	80.7	105.1	170.5
upper 95 %-cl	66.284	101.3	246.8	70.683	162.2	102.0	125.5	190.6

cl: confidence limit

Parameter	96 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg/L]	132.5	158.2	222.3
lower 95 %-cl	105.6	135.1	208.5
upper 95 %-cl	150.7	173.9	236.9

cl: confidence limit

Statistical re-analysis of the available data for **biomass after 48 hours** revealed that no E_bC_{10} could be reliably determined due to the EC value being more than 25 % below the lowest test concentration.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_rC_{50} (*Navicula pelliculosa*, 72 h) = 0.349 mg MITC/L (initial measured)

E_rC_{50} (*Navicula pelliculosa*, 96 h) = 0.468 mg MITC/L (initial measured)

E_yC_{50} (*Navicula pelliculosa*, 72 h) = 0.181 mg MITC/L (initial measured)

E_yC_{50} (*Navicula pelliculosa*, 96 h) = 0.314 mg MITC/L (initial measured)

Endpoints from re-analysis:

Statistical analysis of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_yC_{10} (*Navicula pelliculosa*, 24 h) = 54.9 µg MITC/L (initial measured)

E_yC_{20} (*Navicula pelliculosa*, 24 h) = 87.5 µg MITC/L (initial measured)
E_yC_{20} (*Navicula pelliculosa*, 48 h) = 53.7 µg MITC/L (initial measured)
Statistical analysis of the available data for average specific growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_rC_{10} (*Navicula pelliculosa*, 24 h) = 92.4 µg MITC/L (initial measured)
E_rC_{10} (*Navicula pelliculosa*, 48 h) = 105.7 µg MITC/L (initial measured)
E_rC_{10} (*Navicula pelliculosa*, 72 h) = 144.0 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 24 h) = 160.5 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 48 h) = 160.0 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 72 h) = 196.6 µg MITC/L (initial measured)
Statistical analysis of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be reliably determined:
E_bC_{10} (*Navicula pelliculosa*, 24 h) = 48.8 µg MITC/L (initial measured)
E_bC_{10} (*Navicula pelliculosa*, 72 h) = 92.1 µg MITC/L (initial measured)
E_bC_{10} (*Navicula pelliculosa*, 96 h) = 132.5 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 24 h) = 80.3 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 48 h) = 61.1 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 72 h) = 116.0 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 96 h) = 158.2 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 24 h) = 208.2 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 48 h) = 147.1 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 72 h) = 180.4 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 96 h) = 222.3 µg MITC/L (initial measured)
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analysis of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_yC_{10} (*Navicula pelliculosa*, 24 h, static) = 54.9 µg MITC/L (initial measured)
E_yC_{20} (*Navicula pelliculosa*, 24 h, static) = 87.5 µg MITC/L (initial measured)
E_yC_{20} (*Navicula pelliculosa*, 48 h, static) = 53.7 µg MITC/L (initial measured)
Statistical analysis of the available data for average specific growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_rC_{10} (*Navicula pelliculosa*, 24 h static) = 92.4 µg MITC/L (initial measured)
E_rC_{10} (*Navicula pelliculosa*, 48 h, static) = 105.7 µg MITC/L (initial measured)
E_rC_{10} (*Navicula pelliculosa*, 72 h, static) = 144.0 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 24 h, static) = 160.5 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 48 h, static) = 160.0 µg MITC/L (initial measured)
E_rC_{20} (*Navicula pelliculosa*, 72 h, static) = 196.6 µg MITC/L (initial measured)
Statistical analysis of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be reliably determined:
E_bC_{10} (*Navicula pelliculosa*, 24 h, static) = 48.8 µg MITC/L (initial measured)
E_bC_{10} (*Navicula pelliculosa*, 72 h, static) = 92.1 µg MITC/L (initial measured)
E_bC_{10} (*Navicula pelliculosa*, 96 h, static) = 132.5 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 24 h, static) = 80.3 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 48 h, static) = 61.1 µg MITC/L (initial measured)
E_bC_{20} (*Navicula pelliculosa*, 72 h, static) = 116.0 µg MITC/L (initial measured)

E_bC_{20} (*Navicula pelliculosa*, 96 h, static) = 158.2 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 24 h, static) = 208.2 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 48 h, static) = 147.1 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 72 h, static) = 180.4 µg MITC/L (initial measured)
E_bC_{50} (*Navicula pelliculosa*, 96 h, static) = 222.3 µg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.2/07
Report author:	[redacted]
Report year:	2012c
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Marine Diatom (*Skeletonema costatum*).
Report No.:	703A-107
Document No.:	-
Guidelines followed in study:	OECD Guideline 201 EU Directive 92/69/EEC, Method C.3 US EPA OPPTS Number 850.5400 ISO 14442 Standard
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: MITC Task Force)

Study Summary:

The objective of this study was to determine the toxicity of the test substance, methyl isothiocyanate (MITC), to the marine diatom, *Skeletonema costatum*, over a 96 hour exposure period. The marine diatom, *Skeletonema costatum*, was exposed to five test concentrations and a negative control (culture medium) for 96 hours.
Nominal test concentrations were selected in consultation with the sponsor and were based upon the results of an exploratory range-finding toxicity test. Nominal test concentrations selected were 13, 32, 80, 200 and 500 µg MITC/L. Measured concentrations were determined from samples of test medium collected from each treatment and control group at 0 and 96 hours of the test.
Measured concentrations at test initiation (0 hours) ranged from 82 % to 93 % of nominal concentrations. After 96 hours measured concentrations were in a range of 66 % and 76 %.

Calculated effect concentrations based on measured test concentrations of MITC at test initiation (day 0) were determined to be:

E_rC_{50} (72 h) > 430 µg MITC/L (initial measured)
E_yC_{50} (72 h) = 81 µg MITC/L(initial measured)
E_rC_{50} (96 h) = 218 µg MITC/L(initial measured)
E_yC_{50} (96 h) = 52 µg MITC/L(initial measured)

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 99.7 %
Test species:	Marine diatom (*Skeleonema costatum*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 13, 32, 80, 250 and 500 µg MITC/L
Dilution medium:	Saltwater algal medium containing sodium bicarbonate
Initial cell density, number of replicates:	5000 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	96 hours In-life dates: October 6th to 10th 2011
Test conditions:	temperature: 20 ± 2 °C pH: 8.1 at start, 8.1 – 9.0 at end photoperiod: 14 hours light and 10 hours dark light intensity: 4250 - 4530 lux
Test procedure:	The marine diatom, *Skeletonema costatum*, was exposed to five test concentrations and a negative control (culture medium) for 96 hours. Nominal test concentrations were selected in consultation with the Sponsor and were based upon the results of an exploratory range-finding toxicity test. Nominal test concentrations selected were 13, 32, 80, 200 and 500 µg MITC/L. Three replicate test chambers were maintained in each treatment group and six replicates were maintained in the control group. A single abiotic replicate was maintained at the highest test concentration to assess stability of the test substance under test conditions. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL. Due to the closed-system design, the test solutions were inoculated with approximately 5000 cells/mL rather than 10000 cells/mL as recommended by the OPPTS guideline in order to prevent over-crowding of cells. Samples were collected from each replicate test chamber at approximately 24 hour intervals during the test to determine cell densities, yield and growth rates. Measured concentrations were determined from samples of test medium collected from each treatment and control group at 0 and 96 hours of the test.
Test item analysis:	Samples of the test solutions were collected at approximately 0 and 96 hours to measure concentrations of the test substance. Samples at test initiation were collected from the individual batches of test solution prepared for each treatment and control group prior to distribution into the test chambers. At exposure termination, samples were collected from the pooled replicates from each treatment and control group.
Observations:	Test medium samples were collected from each replicate of the treatment and control groups for the determination of algal cell densities. Samples were collected at approximately 24 hour intervals during the 96 hour exposure using a syringe and were held for a maximum of four days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts were performed using an electronic particle counter.

Statistical evaluation: The calculation of yield, growth rates and percent inhibition values, as well as all statistical analyses, were conducted using "The SAS System for Windows, Version 8.2". Yield and growth rates were used to calculate percent inhibition values relative to the control over the 96 hour exposure period. E_yC_{50} and E_rC_{50} values (i.e., the theoretical toxicant concentrations that would produce a 50 % reduction in yield and growth rate, respectively) were calculated, when possible, for each 24 hour interval of the exposure period. No-observed-adverse-effect-concentrations (NOAEC) were determined at 72 and 96 hours through statistical evaluation of yield and growth rates, as well as examination of the concentration-response pattern.

Findings:

Analytical results: Measured concentrations on day 0 ranged from 82 to 93 % of nominal. Measured concentrations declined by day 4, and ranged from 66 to 76 % of nominal.

The biological results of the study are based on day 0 measured concentrations of 12, 30, 68, 165 and 430 µg MITC/L, representing 93, 92, 84, 82 and 86 % of the target nominal test concentrations.

The measured concentrations of MITC in the abiotic control was 430 µg MITC/L at test initiation and 373 µg MITC/L at test termination.

Results of measured MITC concentrations are presented in the table below.

Inhibition of biomass and growth: After 72 hours of exposure, inhibition of yield in the 12, 30, 68, 165 and 430 µg MITC/L treatment groups was 0 %, 5 %, 49 %, 79 % and 81 %, respectively, relative to the negative control. Inhibition of growth rate in the 12, 30, 68, 165 and 430 µg MITC/L treatment groups was 0 %, 2 %, 18 %, 41 % and 43 %, respectively, relative to the negative control. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 68, 165 and 430 µg MITC/L treatment groups. Consequently, the 72 hour NOAEC for yield and growth rate was 30 µg MITC/L.

After 96 hours of exposure, inhibition of yield in the 12, 30, 68, 165 and 430 µg MITC/L treatment groups was 4 %, 1 %, 70 %, 94 % and 93 %, respectively, relative to the negative control. Inhibition of growth rate in the 12, 30, 68, 165 and 430 µg MITC/L treatment groups was 1 %, 0 %, 26 %, 58 % and 56 %, respectively, relative to the negative control. Dunnett's test indicated that there were significant reductions ($p \leq 0.05$) in yield and growth rate in the 68, 165 and 430 µg MITC/L treatment groups. Consequently, the 96 hour NOAEC for yield and growth rate was 30 µg MITC/L.

After 96 hours of exposure, there were flocculation and aggregation of cells in the negative control and all treatment groups. Adherence of cells to the test chambers was not noted in any of the test chambers. There were no noticeable changes in cell morphology in any of the test concentrations when

compared to the control replicates during the microscopic examinations of the cells.
Details are provided in the table below.

Table B.2.9.2.2-89: Measured concentrations of MITC in acidified saltwater $NaHCO_3$ Algal Medium Samples at test initiation (day 0) and at test termination (day 4) of the Algal Growth Inhibition Test with *Skeletonema costatum*

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)				% of nominal concentration
	Day 0	Day 4	% of day 0	Mean	
Control	< LOQ[1]	< LOQ[1]	-	-	-
13	12.1	8.58	70.9	10.3	79.5
32	29.5	23.4	79.3	26.5	82.7
80	67.6	60.7*	89.8	64.2	80.2
200	165	138	83.6	152	75.8
500	430	343	79.8	387	77.3

[1] The limit of quantitation (LOQ) was 5.00 µg a.is./L, calculated as the product of the concentration of the lowest calibration standard (10.0 µg a.s./L) and the dilution factor of the matrix blank samples (0.500). The limit of detection (LOD) was 3.09 µg a.s./L.
** Extrapolated value. Peak area was slightly greater than the highest calibration standard. Measurement otherwise considered reliable.*

Table B.2.9.2.2-90: Results of inhibition of growth of *Skeletonema costatum*, exposed to MITC for 96 hours under static test conditions, based on measured concentrations of MITC at test initiation (day 0)

Duration of Exposure	Yield		Growth	
	E_yC_{50} (µg MITC/L)	95 % Confidence Interval (µg MITC/L)[1]	E_rC_{50} (µg MITC/L)	95 % Confidence Interval (µg MITC/L)[1]
24 hours	> 430	n/a[2]	> 430	n/a[2]
48 hours	114	86 - 153	> 430	n/a[2]
72 hours	81	54 - 123	> 430	n/a[2]
96 hours	52	33 - 83	218	147 - 334
	NOAEC (µg MITC/L)		NOAEC (µg MITC/L)	
72 hours	30		30	
96 hours	30		30	

[1] E_yC_{50} and E_rC_{50} values and their 95 % confidence intervals were calculated using non-linear regression (Bruce and Versteeg 1992, Environmental Toxicology and Chemistry 11: 1485-1494) with replicate data (yield or growth rate) and day 0 measured test concentrations.
[2] 95 % confidence intervals were not able to be calculated.

Assessment and conclusions:

The marine diatom, *Skeletonema costatum*, was exposed to a geometric series of five concentrations of MITC ranging from 12 to 430 µg MITC/L based on measured concentrations of MITC at test initiation (day 0). Effects were evaluated based on yield and growth rate. The 72 and 96 hour E_rC_{50} values based on growth rate, were > 430 µg MITC/L (95 % confidence interval unable to be calculated) and 218 (95 % confidence interval 147 to 334) µg MITC/L, respectively. The 72 and 96 hour E_yC_{50} values based on yield, were 81 (95 % confidence interval 54 to 123) µg MITC/L and 52 (95 % confidence interval 33 to 83) µg MITC/L. The 72 and 96 hour no-observed-adverse-effect concentration (NOAEC), based on effects on yield and growth rate was 30 µg MITC/L for both intervals.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):

The study is acceptable.
Endpoints:
E_rC_{50} (*Skeletonema costatum*, 72 h) > 0.430 mg MITC/L (initial measured)
E_rC_{50} (*Skeletonema costatum*, 96 h) = 0.218 mg MITC/L (initial measured)
E_yC_{50} (*Skeletonema costatum*, 72 h) = 0.081 mg MITC/L (initial measured)
E_yC_{50} (*Skeletonema costatum*, 96 h) = 0.052 mg MITC/L (initial measured)
Analytical method:
Method validation not fully acceptable according to the guidance SANCO/3029/99 rev. 4 on analytical validation, but is accepted as fit for purpose.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: 35)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 19.1 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 10 % (measured: 2.5 %)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
No further information on the analytical method used.
E_rC_{50} (*Skeletonema costatum*, 72 h, static) > 0.430 mg MITC/L (initial measured)
E_rC_{50} (*Skeletonema costatum*, 96 h, static) = 0.218 mg MITC/L (initial measured)
E_yC_{50} (*Skeletonema costatum*, 72 h, static) = 0.081 mg MITC/L (initial measured)
E_yC_{50} (*Skeletonema costatum*, 96 h, static) = 0.052 mg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.2/08.

Data point:	KCA 8.2.6.2/08
Report author:	[redacted]
Report year:	2020b
Report title:	Methyl isothiocyanate (MITC): A 96-hour toxicity test with the Marine Diatom (*Skeletonema costatum*) – Statistical Re-analysis.
Report No.:	CEA.2139
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable

Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report from Wildlife International Ltd., Study code 703A-107 ([redacted] 2012; KCA 8.2.6.2/07), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the marine diatom *Skeletonema costatum* did not provide estimates of the EC_{10} or EC_{20} for the response variables evaluated as part of the original study. In addition, the biomass integral over the total growth curve (area under the growth curve) was not calculated as part of the original study and estimates of the EC_x were not provided. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal measured concentrations of MITC at 13, 32, 80, 200 and 500 µg a.s./L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 82 % to 93 % of the nominal values from test initiation and from 66 % to 76 % in samples on day 4 of the test. In line with the original study, the initial mean measured test concentrations were used for this analysis, the concentrations were 12, 30, 68, 165 and 430 µg a.s./L representing 93, 92, 84, 82 and 86 % of the nominal test concentrations.

The following parameters were analysed statistically:

- Yield (Y)
- Average specific growth rate (µ)
- Biomass (b)

Statistical analyses of the available data for **yield** revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table B.2.9.2.2-91: Summary of effects on *Skeletonema costatum*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for yield, based on initial measured values

Parameter	48 hours		72 hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [µg/L]	16.3	31.9	24.6	37.2
lower 95 %-cl	9.2	21.3	14.9	26.0
upper 95 %-cl	23.9	42.5	33.2	47.0

cl: confidence limits

Statistical re-analysis of the available data for **yield after 24 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was non-significant, p(F) = 0.998, and the slope function was 0.000. As a result, the EC_{10} and EC_{20} values could not be determined from this data.

Statistical re-analysis of the available data for **yield after 96 hours** revealed that no E_yC_{10} or E_yC_{20} could be reliably calculated. The relationship between dose and response was non-significant, p(F) = 0.288, and the slope function was 1.331. As a result, the EC_{10} and EC_{20} values could not be determined from this data.

Statistical analyses of the available data for **average specific growth rate** revealed that the following E_rC_{10} and E_rC_{20} values were reliably calculated:

Table B.2.9.2.2-92: Summary of effects on *Skeletonema costatum*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for growth rate, based on initial measured values

Parameter	48 hours		72 hours		96 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [µg/L]	34.4	81.9	35.1	83.8	27.1	55.4
lower 95 %-cl	20.5	60.0	23.5	65.8	18.3	42.4
upper 95 %-cl	48.7	103.3	46.9	101.5	36.2	68.1

cl: confidence limit

Statistical re-analysis of the available data for **average specific growth rate after 24 hours** revealed that no E_rC_{10} or E_rC_{20} could be reliably calculated. The relationship between dose and response was non-significant, p(F) = 0.998, and the slope function was 0.000. As a result, the EC_{10} and EC_{20} values could not be determined from this data.

Statistical analyses of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-93: Summary of effects on *Skeletonema costatum*, exposed to MITC for 96 hours under static test conditions, statistical re-analysis for biomass, based on initial measured values

Parameter	48 hours		72 hours			96 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg/L]	57.9	122.8	21.0	40.2	139.1	24.7	35.8	72.4
lower 95 %-cl	20.1	67.7	15.3	32.1	122.4	20.1	30.7	66.3
upper 95 %-cl	94.1	172.4	26.9	48.2	159.1	29.0	40.4	79.1

cl: confidence limit

Statistical re-analysis of the available data for **biomass after 24 hours** revealed that no E_bC_{10}, E_bC_{20} or E_bC_{50} could be reliably calculated. The relationship between dose and response was non-significant, p(F) = 0.931, and the slope function was 0.000. As a result, the EC_{10}, EC_{20} and EC_{50} values could not be determined from this data.

Statistical re-analysis of the available data for **biomass after 48 hours** revealed that no E_bC_{50} could be reliably calculated. The calculated E_bC_{50} of 517.168 µg/L was not considered to be reliable as it was more than 25 % above the highest test concentration.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_rC_{50} (*Skeletonema costatum*, 72 h) > 0.430 mg MITC/L (initial measured)

E_rC_{50} (*Skeletonema costatum*, 96 h) = 0.218 mg MITC/L (initial measured)

E_yC_{50} (*Skeletonema costatum*, 72 h) = 0.081 mg MITC/L (initial measured)

E_yC_{50} (*Skeletonema costatum*, 96 h) = 0.052 mg MITC/L (initial measured)

Endpoints from re-analysis:

Statistical analyses of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:

E_yC_{10} (*Skeletonema costatum*, 48 h) = 16.3 µg MITC/L (initial measured)
E_yC_{10} (*Skeletonema costatum*, 72 h) = 24.6 µg MITC/L (initial measured)
E_yC_{20} (*Skeletonema costatum*, 48 h) = 31.9 µg MITC/L (initial measured)
E_yC_{20} (*Skeletonema costatum*, 72 h) = 37.2 µg MITC/L (initial measured)
Statistical analyses of the available data for average specific growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_rC_{10} (*Skeletonema costatum*, 48 h) = 34.4 µg MITC/L (initial measured)
E_rC_{10} (*Skeletonema costatum*, 72 h) = 35.1 µg MITC/L (initial measured)
E_rC_{10} (*Skeletonema costatum*, 96 h) = 27.1 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 48 h) = 81.9 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 48 h) = 83.8 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 96 h) = 55.4 µg MITC/L (initial measured)
Statistical analyses of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be reliably determined:
E_bC_{10} (*Skeletonema costatum*, 48 h) = 57.9 µg MITC/L (initial measured)
E_bC_{10} (*Skeletonema costatum*, 72 h) = 21.0 µg MITC/L (initial measured)
E_bC_{10} (*Skeletonema costatum*, 96 h) = 24.7 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 48 h) = 122.8 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 72 h) = 40.2 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 96 h) = 35.8 µg MITC/L (initial measured)
E_bC_{50} (*Skeletonema costatum*, 72 h) = 139.1 µg MITC/L (initial measured)
E_bC_{50} (*Skeletonema costatum*, 96 h) = 72.4 µg MITC/L (initial measured)
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analyses of the available data for yield revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_yC_{10} (*Skeletonema costatum*, 48 h, static) = 16.3 µg MITC/L (initial measured)
E_yC_{10} (*Skeletonema costatum*, 72 h, static) = 24.6 µg MITC/L (initial measured)
E_yC_{20} (*Skeletonema costatum*, 48 h, static) = 31.9 µg MITC/L (initial measured)
E_yC_{20} (*Skeletonema costatum*, 72 h, static) = 37.2 µg MITC/L (initial measured)
Statistical analyses of the available data for average specific growth rate revealed that the following EC_{10} and EC_{20} values could be reliably determined:
E_rC_{10} (*Skeletonema costatum*, 48 h, static) = 34.4 µg MITC/L (initial measured)
E_rC_{10} (*Skeletonema costatum*, 72 h, static) = 35.1 µg MITC/L (initial measured)
E_rC_{10} (*Skeletonema costatum*, 96 h, static) = 27.1 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 48 h, static) = 81.9 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 48 h, static) = 83.8 µg MITC/L (initial measured)
E_rC_{20} (*Skeletonema costatum*, 96 h, static) = 55.4 µg MITC/L (initial measured)
Statistical analyses of the available data for biomass revealed that the following EC_{10}, EC_{20} and EC_{50} values could be reliably determined:
E_bC_{10} (*Skeletonema costatum*, 48 h, static) = 57.9 µg MITC/L (initial measured)
E_bC_{10} (*Skeletonema costatum*, 72 h, static) = 21.0 µg MITC/L (initial measured)
E_bC_{10} (*Skeletonema costatum*, 96 h, static) = 24.7 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 48 h, static) = 122.8 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 72 h, static) = 40.2 µg MITC/L (initial measured)
E_bC_{20} (*Skeletonema costatum*, 96 h, static) = 35.8 µg MITC/L (initial measured)

E_bC_{50} (*Skeletonema costatum*, 72 h, static) = 139.1 µg MITC/L (initial measured)
E_bC_{50} (*Skeletonema costatum*, 96 h, static) = 72.4 µg MITC/L (initial measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.6.2/09
Report author:	[redacted]
Report year:	2019
Report title:	Methyl isothiocyanate (MITC) – Effect on *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test + Amendment No. 1.
Report No.:	IES Study 20180139
Document No.:	-
Guidelines followed in study:	OECD 201 (2011) Method C.3 of Commission Regulation (EU) No. 2016/266
Deviations from current test guideline:	Deviations from current OECD guideline 201 (2011): None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities (exception: pre-test for verification of the stability of the test item and range-finding tests (non-GLP))
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: Kanesho Soil Treatment, letter of access by Taminco is included, study may be used by Taminco in Europe only) (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

In a 72 hour acute toxicity study, the cultures of *Anabaena flos-aquae* (strain CCAP 1403/13A) were exposed to MITC at nominal concentrations of 10, 29, 84, 244, 707 and 2051 µg/L under static conditions in accordance with the OECD TG 201 and the Commission Regulation (EU) No 2016/266, C.3. The NOEC and EC_{50} values based on growth rate and yield were 84 and 375 µg/L (growth rate) and 10 and 181 µg/L (yield), respectively. The % growth inhibition in the treated algal culture as compared to the control ranged from 2.5 % at 10 µg/L MITC to 99.9 % at 2051 µg/L MITC.
There were no compound related phytotoxic effects.
This toxicity study is classified as acceptable and satisfies the guideline requirements for a 72 hour algal growth inhibition test.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 %
Test species:	Blue-green algae (*Anabaena flos-aquae*)
Type of test:	Static toxicity test
Applied concentrations:	Nominal test concentrations: 0 (control), 10, 26, 64, 160, 400 and 1000 µg MITC/L
Dilution medium:	AAP algal medium

Initial cell density, number of replicates:	10000 cells/mL, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	72 hours
Test conditions:	temperature: 23 °C pH: 7.0 – 7.1 at start, 7.4 - 7.5 at end photoperiod: continuous illumination light intensity: 48 – 51 µE s^{-1} m^{-2}
Test procedure:	The test organisms used for the study was the cyanobacterium ("blue alga") *Anabaena flos-aquae*. Based on the results of a range-finding test, following nominal test concentrations were tested: 10, 29, 84, 244, 707 and 2051 µg MITC/L. A control (test water without test item) was tested in parallel. The test design included three replicates per test concentration and six replicates for the control. Additionally, two replicates per treatment were prepared for analytical measurements at 24 and 48 hours, which were incubated in parallel to the test. The test was started using a nominal algal cell density of 10000 cells/mL. A static test design was applied. The duration of the test was 72 hours.
Test item analysis:	For measurement of the actual concentrations of the test item, duplicate samples without algae were taken from the test media of all test concentrations and the control at the start of the test. About 20 g of sodium chloride were added to the test samples subsequently to sampling. Then, the samples (50 mL) were extracted with 10 mL of internal standard solution. The organic phase was separated and frozen (at about -20 °C) immediately after extraction. The maximum storage time of the extracts until analysis was 16 days. The concentrations of the test item Methyl isothiocyanate (MITC) were determined in one of the duplicate samples from the control and all concentrations from all sampling dates. Samples were analysed by gas chromatography with mass spectrometric detection (GC/MS).
Observations:	A small volume (3 mL per sampling) of the algal suspension was withdrawn daily from each test flask for the measurement of the biomass, and was not replaced. Prior to the measurements, this aliquot was sonicated in order to break up the filaments of the algae to single cells. The algal biomass in the samples was determined by fluorescence measurement. The measurements were performed at least in duplicate at an excitation of 620 nm and emission of 655 nm. At the end of the test, a sample was taken from the control and from the nominal test concentration of 244 µg MITC/L to determine a potential influence of the test item on the algal cells. The shape and size of the algal cells were visually inspected. This test concentration was chosen because the algal cell density at the two highest nominal concentrations of 707 and 2051 µg MITC/L as too low for a reliable examination. The experimental conditions were monitored. The light intensity was measured at the start of the test. The temperature in the incubator was monitored and recorded continuously. The pH was measured and recorded in each treatment at the start and end of the

test. The appearance of the test media was visually controlled and recorded daily.

Statistical evaluation: The 72 hour EC_{10}, EC_{20} and EC_{50} values for the inhibition of average growth rate and yield and their 95 % confidence intervals were calculated by Probit Analysis using linear maximum likelihood regression.
For the determination of the LOEC and NOEC, the average growth rate and yield at the test concentrations were compared to the control values by Williams' t-test, or Welch t-test, where appropriate.
Statistital analyses were performed using ToxRat Professional®.

Findings:

Analytical results: The measured concentrations of Methyl isothiocyanate (MITC) in the test media of all the test concentrations were between 86 and 106 % of the nominal values at the start of the test. After 24 hours and 48 hours the measured concentrations ranged between 81 to 98 % and 82 to 98 % of the nominal values, respectively. At the end of the test the measured concentrations of the test item in the test media of all the test concentrations were between 80 and 89 % of the nominal values, except for one, which was 77 %.
Thus, the correct dosage and the stability in the test media over the test period of 72 hours of the test item Methyl isothiocyanate (MITC) was confirmed. Therefore, the biological results were related to the nominal concentrations of the test item.

Inhibition of biomass and growth: <u>Growth rate:</u>
At the test item concentrations of 29 and 84 µg MITC/L the mean inhibition compared to the control was 3.3 % and 6.3 %, respectively. These values were statistically significant (results of Williams' t-test, one-sided smaller, $\alpha = 0.05$) and this significance was caused by the very low variability between replicates within these test concentrations and the control. However, this statistically significant finding was not considered as a biologically relevant toxic effect, since the mean inhibition compared to the control was below 10 %. Moreover, the 72 hour E_rC_{10} for growth rate was calculated to be 173 µg MITC/L (95 % confidence limits: 163 – 183 µg/L).
At the higher concentrations of 244 to 2051 µg MITC/L, the mean inhibition compared to the control was in the range of 23 % to 99 % and was statistically significantly different from the control. Therefore, the NOEC for growth rate was determined to be at the test concentration of 84 µg MITC/L.
The 72 hour E_rC_{20} and E_rC_{50} were calculated to be 226 µg MITC/L (95 % confidence limits: 215 – 235 µg/L) and 375 µg MITC/L (95 % confidence limits: 363 – 388 µg/L), respectively.
<u>Yield:</u>
At the test item concentration of 10 µg MITC/L the mean inhibition compared to the control was 8.9 %. These values were statistically significant (results of Williams t-test, one-sided smaller, $\alpha = 0.05$) and this significance was caused by the very

low variability between replicates within this test concentration and the control. However, this statistically significant finding was not considered as a biologically relevant toxic effect, since the mean inhibition compared to the control was below 10 %. Moreover, the 72 hour E_yC_{10} for yield was calculated to be 51 µg MITC/L (95 % confidence limits: 44 – 57 µg/L).
At the higher concentrations of 29 to 2051 µg MITC/L, the mean inhibition compared to the control was in the range of 11 % to 100 % and was statistically significantly different from the control. Therefore, the NOEC for yield was determined to be at the test concentration of 10 µg MITC/L.
The 72 hour E_yC_{20} and E_yC_{50} were calculated to be 79 µg MITC/L (95 % confidence limits: 71 – 86 µg/L) and 181 µg MITC/L (95 % confidence limits: 170 – 193 µg/L), respectively.

The microscopic examination of the algal cells at the end of the test showed no difference between the algae growing at the nominal test concentration of 244 µg MITC/L and the algal cells in the control. The shape and size of the algal cells were obviously not affected by the test item up to at least this test concentration.
No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the test period.

Table B.2.9.2.2-94: Daily measured concentrations of MITC in the test media of the Algal Growth Inhibition Test with *Anabaena flos-aquae*

Nominal concentration [µg/L]		**0 (control)**	**10**	**29**	**84**	**244**	**707**	**2051**
0 hours	Measured concentration	< LOD	8.59	27.2	80.1	232	747	1832
	% of nominal (ref. to mean)	n.a.	86	94	95	95	106	89
24 hours	Measured concentration	< LOD	8.91	25.9	79.3	225	691	1670
	% of nominal (ref. to mean)	n.a.	89	89	94	92	98	81
48 hours	Measured concentration	< LOD	8.19	25.5	77.4	226	695	1747
	% of nominal (ref. to mean)	n.a.	82	88	92	93	98	85
72 hours	Measured concentration	< LOD	7.73	23.3	73.2	209	628	1752
	% of nominal (ref. to mean)	n.a.	77	80	87	86	89	85

Table B.2.9.2.2-95: Effect on Biomass of algae of *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Biomass of Algae* (mean ± SD)		
	24 hours	48 hours	72 hours
Control	6.4 ± 0.27	22 ± 1.0	70 ± 3.0
10	6.4 ± 0.83	21 ± 0.85	64 ± 2.4
29	7.7 ± 1.1	21 ± 2.2	62 ± 6.3
84	5.9 ± 0.37	19 ± 1.2	56 ± 2.5
244	2.7 ± 2.0	14 ± 0.75	30 ± 0.70
707	2.2 ± 0.20	2.5 ± 0.21	3.2 ± 0.47
2051	1.7 ± 0.08	1.8 ± 0.24	2.0 ± 0.23

** The biomass was determined by fluorescence measurement (mean of duplicate measurements per replicate) and is given as relative fluorescence units (x 10^4). At the start of the test, the initial cell density was10000 algal cells/mL, corresponding to 1.88 x 10^4 relative fluorescence units.*

Table B.2.9.2.2-96: Effect on Average growth rates (µ) and percent inhibition for *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Average Growth Rate µ [day^{-1}] and Inhibition Ir [%]					
	0-24 hours		0-48 hours		0-72 hours	
	µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]
Control	1.221	0.0	1.223	0.0	1.204	0.0
10	1.211	0.8	1.210	1.0	1.174	2.5
29	1.406	-15.2	1.192	2.5	1.164(*)	3.3
84	1.149	5.9	1.159(*)	5.3	1.128(*)	6.3
244	0.172	85.9	0.999*	18.3	0.925*	23.1
707	0.150*	87.7	0.139*	88.6	0.170*	85.9
2051	-0.126*	110.3	-0.025*	102.1	0.012*	99.0

** Mean value statistically significantly lower than in the control (according to a Williams' t-test, one-sided smaller, α = 0.05).*
(*) Mean value statistically significantly different from the control due to very low variability of results, however not estimated as a biologically relevant toxic effect (according to Williams t-test, one-sided smaller, α = 0.05).

Table B.2.9.2.2-97: Effect on section-by-section growth rates (µ) and percent inhibition for *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Section-by-Section Growth Rates [day^{-1}] and Inhibition Ir [%]					
	0-24 hours		24-48 hours		48-72 hours	
	µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]	µ [day^{-1}]	Ir [%]
Control	1.221	0.0	1.226	0.0	1.165	0.0
10	1.406	-15.2	0.978	20.2	1.107	5.0
29	1.406	-15.2	0.978	20.2	1.107	5.0
84	1.149	5.9	1.169	4.6	1.066	8.5
244	0.172	85.9	1.827	-49.0	0.777	33.3
707	0.150	87.7	0.128	89.6	0.231	80.2
2051	-0.126	110.3	0.075	93.9	0.086	92.6

Table B.2.9.2.2-98: Effect Yield (Y) and percent inhibition for *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions

Nominal Test Item Concentration [µg/L]	Yield Y (× 10^4) and Inhibition Iy [%]					
	0-24 hours		0-48 hours		0-72 hours	
	Y	Iy [%]	Y	Iy [%]	Y	Iy [%]

Control	4.50	0.0	19.87	0.0	67.87	0.0
10	4.47	0.7	19.33	2.8	61.85(*)	8.9
29	5.86	-30.0	18.63	6.3	60.15*	11.4
84	4.07	9.7	17.26*	13.2	53.66*	20.9
244	0.79*	82.4	12.03*	39.5	28.35*	58.2
707	0.31*	93.1	0.61*	96.9	1.27*	98.1
2051	-0.22*	104.9	-0.08*	100.4	0.08*	99.9

** Mean value statistically significantly lower than in the control (according to a Williams' t-test, one-sided smaller, α = 0.05).*
(*) Mean value statistically significantly different from the control due to very low variability of results, however not estimated as a biologically relevant toxic effect (according to Williams t-test, one-sided smaller, α = 0.05).

Assessment and conclusions:

The impact of the test item Methyl isothiocyanate (MITC) on the growth of the cyanobacterium ("blue algae") *Anabaena flos-aquae* in a 72 hour static test is listed below.
According to the growth rate, the 72 hour EC_{10} was calculated to be 173 µg MITC/L (95 % confidence limits: 163 – 183 µg/L); the 72 hour EC_{20} was calculated to be 226 µg MITC/L (95 % confidence limits: 215 – 235 µg/L); the 72 hour EC_{50} was calculated to be 375 µg MITC/L (95 % confidence limits: 363 – 388 µg/L); the 72 hour NOEC was calculated as 84 µg MITC/L and the 72 hour LOEC was calculated as 244 µg MITC/L.
With regard to yield, the 72 hour EC_{10} was calculated to be 51 µg MITC/L (95 % confidence limits: 44 – 57 µg/L); the 72 hour EC_{20} was calculated to be 79 µg MITC/L (95 % confidence limits: 71 – 86 µg/L); the 72 hour EC_{50} was calculated to be 181 µg MITC/L (95 % confidence limits: 170 – 193 µg/L); the 72 hour NOEC was calculated as 10 µg MITC/L and the 72 hour LOEC was calculated as 29 µg MTIC/L.
The results are based on nominal concentrations of the test item.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 375 µg MITC/L (nominal) (95 % confidence limits: 363 – 388 µg/L)
E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 181 µg MITC/L (nominal) (95 % confidence limits: 170 – 193 µg/L)
Analytical method:
Method validation fully compliant with the guidance SANCO/3029/99 rev. 4 on analytical validation and therefore acceptable.
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 375 µg MITC/L (nominal) (95 % confidence limits: 363 – 388 µg/L)
E_rC_{20} (*Anabaena flos-aquae*, 72 h, static) = 226 µg MITC/L (nominal) (95 % confidence limits: 215 – 235 µg/L)
E_rC_{10} (*Anabaena flos-aquae*, 72 h, static) = 173 µg MITC/L (nominal) (95 % confidence limits: 163 – 183 µg/L)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.

The validity criteria of OECD Guideline 201 were met:

- the biomass in the controls should have increased exponentially by a factor of at least 16 within the 72 hour test period (measured: 37)
- the mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35 % (measured: 4.0 %)
- the coefficient of variation of average specific growth rates during the whole test period in controls must not exceed 10 % (measured: 1.2 %)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 201 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/72 (Taminco) and KCA 4.1.2/24 (Lainco), for further details).
E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 0.375 mg MITC/L (nominal) (95 % confidence limits: 0.363 – 0.388 mg/L)
E_rC_{20} (*Anabaena flos-aquae*, 72 h, static) = 0.226 mg MITC/L (nominal) (95 % confidence limits: 0.215 – 0.235 mg/L)
E_rC_{10} (*Anabaena flos-aquae*, 72 h, static) = 0.173 mg MITC/L (nominal) (95 % confidence limits: 0.163 – 0.183 mg/L)
E_yC_{50} (*Anabaena flos-aquae*, 72 h, static) = 0.181 mg MITC/L (nominal) (95 % confidence limits: 0.170 – 0.193 mg/L)
E_yC_{20} (*Anabaena flos-aquae*, 72 h, static) = 0.079 mg MITC/L (nominal) (95 % confidence limits: 0.071 – 0.086 mg/L)
E_yC_{10} (*Anabaena flos-aquae*, 72 h, static) = 0.051 mg MITC/L (nominal) (95 % confidence limits: 0.044 – 0.057 mg/L)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.6.2/10.

Data point:	KCA 8.2.6.2/10
Report author:	[redacted]
Report year:	2020e
Report title:	Methyl isothiocyanate (MITC) – Effect on *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test + Amendment No. 1 – Statistical Re-analysis.
Report No.:	CEA.2144
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted July 28, 2011.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical analysis)

Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The original report from Innovative Environmental Services (IES) Ltd., Study 20180139 ([redacted], 2019; KCA 8.2.6.2/09), for the toxicity of Methyl isothiocyanate (MITC) on the growth of the freshwater algae *Anabaena flos-aquae* did not provide estimates of the EC_{10}, EC_{20} or EC_{50} for the biomass integral over the total growth curve (area under the growth curve). Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal measured concentrations of MITC at 10, 29, 84, 244, 707 and 2051 µg/L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 86 % to 106 % of the nominal values from test initiation and from 77 % to 89 % in samples at the end of the test. In line with the original study, the nominal values were used for this analysis.

The following parameters were analysed statistically:

- Biomass (b)

Statistical analyses of the available data for **biomass** revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

Table B.2.9.2.2-99: Summary of effects on *Anabaena flos-aquae*, exposed to MITC for 72 hours under static test conditions, statistical re-analysis for biomass, based on nominal values

Parameter	24 hours			48 hours			72 hours		
	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value [µg/L]	85	107	167	84	118	227	67	99	208
lower 95 %-cl	18	33	100	63	95	202	58	89	195
upper 95 %-cl	127	150	230	103	138	254	75	108	221

cl: confidence limit

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Endpoints:

E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 375 µg MITC/L (nominal) (95 % confidence limits: 363 – 388 µg/L)

E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 181 µg MITC/L (nominal) (95 % confidence limits: 170 – 193 µg/L)

Endpoints from re-analysis:

Statistical analysis of the available data for biomass revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:

E_bC_{10} (*Anabaena flos-aquae*, 24 h) = 85 µg MITC/L (nominal)

E_bC_{10} (*Anabaena flos-aquae*, 48 h) = 84 µg MITC/L (nominal)

E_bC_{10} (*Anabaena flos-aquae*, 72 h) = 67 µg MITC/L (nominal)

E_bC_{20} (*Anabaena flos-aquae*, 24 h) = 107 µg MITC/L (nominal)

E_bC_{20} (*Anabaena flos-aquae*, 48 h) = 118 µg MITC/L (nominal)

E_bC_{20} (*Anabaena flos-aquae*, 72 h) = 99 µg MITC/L (nominal)

E_bC_{50} (*Anabaena flos-aquae*, 24 h) = 167 µg MITC/L (nominal)

E_bC_{50} (*Anabaena flos-aquae*, 48 h) = 227 µg MITC/L (nominal)

E_bC_{50} (*Anabaena flos-aquae*, 72 h) = 208 µg MITC/L (nominal)
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
E_rC_{50} (*Anabaena flos-aquae*, 72 h, static) = 375 µg MITC/L (nominal) (95 % confidence limits: 363 – 388 µg/L)
E_rC_{20} (*Anabaena flos-aquae*, 72 h, static) = 226 µg MITC/L (nominal) (95 % confidence limits: 215 – 235 µg/L)
E_rC_{10} (*Anabaena flos-aquae*, 72 h, static) = 173 µg MITC/L (nominal) (95 % confidence limits: 163 – 183 µg/L)

Assessment and conclusion by the RMS:
The study is still considered acceptable.
Endpoints from re-analysis:
Statistical analysis of the available data for biomass revealed that the following E_bC_{10}, E_bC_{20} and E_bC_{50} values were reliably calculated:
E_bC_{10} (*Anabaena flos-aquae*, 24 h, static) = 85 µg MITC/L (nominal)
E_bC_{10} (*Anabaena flos-aquae*, 48 h, static) = 84 µg MITC/L (nominal)
E_bC_{10} (*Anabaena flos-aquae*, 72 h, static) = 67 µg MITC/L (nominal)
E_bC_{20} (*Anabaena flos-aquae*, 24 h, static) = 107 µg MITC/L (nominal)
E_bC_{20} (*Anabaena flos-aquae*, 48 h, static) = 118 µg MITC/L (nominal)
E_bC_{20} (*Anabaena flos-aquae*, 72 h, static) = 99 µg MITC/L (nominal)
E_bC_{50} (*Anabaena flos-aquae*, 24 h, static) = 167 µg MITC/L (nominal)
E_bC_{50} (*Anabaena flos-aquae*, 48 h, static) = 227 µg MITC/L (nominal)
E_bC_{50} (*Anabaena flos-aquae*, 72 h, static) = 208 µg MITC/L (nominal)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for algae.

Data point:	KCA 8.2.7/01
Report author:	[redacted]
Report year:	2002
Report title:	Effect of Methyl isothiocyanate (Metabolite of BAS 002 N, Dazomet) on the Growth of *Lemna gibba*.
Report No.:	121437
Document No.:	2002/1006181
Guidelines followed in study:	OECD draft guideline: "*Lemna* sp., Growth Inhibition Test", Dec. 1999 OPPTS Number 850.5400, ASTM E1415-91, EPA, Subdiv. J, 132-2
Deviations from current test guideline:	Deviations from current OECD guideline 221 (2006): None
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

In a semi-static toxicity test, the duckweed *Lemna gibba* was exposed for 7 days to a series of six concentrations of MITC ranging from 0.01 to 2.863 mg a.s./L. For each test concentration three replicates were prepared, 6 for the control. One plant with four fronds and two plants with three fronds were added impartially to each vessel under axenic conditions giving a total number of 10 fronds at test initiation. Frond production and appearance were recorded on days 3, 5 and 7. Observations on the appearance of the fronds included necrosis, chlorosis, changes in plant size or shape and root growth. The test substance degraded rapidly during the course of the test. Therefore, water renewal was done 3 days after test initiation. Analytical recoveries at test initial and after water renewal ranged from 79 % to 178 % of nominal. The following biological results are based on mean measured initial concentrations of MITC, except for the lowest concentration which was based on nominal.

E_rC_{50} (7 day) = 1.18 mg a.s./L (95 % limits: 1.12 - 1.23 mg a.s./L)
E_rC_{10} (7 day) = 0.26 mg a.s./L (95 % limits: 0.24 - 0.29 mg a.s./L)
E_bC_{50} (7day) = 0.59 mg a.s./L (95 % limits: 0.56 - 0.62 mg a.s./L)
E_bC_{10} (7day) = 0.10 mg a.s./L (95 % limits: 0.09 - 0.11 mg a.s./L)
NOEC = 0.09 mg a.s./L, LOEC = 0.269 mg a.s./L,
MATC (maximum acceptable toxicant concentration) = 0.156 mg a.s./L

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 408208/1, chemical purity: 99.6 %
Test species:	Duckweed (*Lemna gibba*)
Type of test:	Semi-static toxicity test (medium renewal after 3 days)
Applied concentrations:	Nominal test concentrations: 0 (control), 0.01, 0.031, 0.096, 0.298, 0.924 and 2.863 mg MITC/L
Dilution medium:	20x AAP medium
Number of organisms, number of replicates:	One plant with 4 fronds and two plants with 3 fronds, giving a total number of 10 fronds per replicate at test initiation, 6 replicates for the control and 3 replicates per treatment group
Time of exposure:	7 days
Test conditions:	temperature: 26.0 °C pH: 7.50 – 7.59 at start, 7.93 – 9.02 at end photoperiod: continuous illumination light intensity: 7970 lux
Test procedure:	The duckweed *Lemna gibba* G3 was exposed for 7 days to nominal concentrations of 0, 0.01, 0.031, 0.096, 0.298, 0.924 and 2.863 mg a.s./L. For each test concentration three replicates were prepared, 6 for the control. One plant with four fronds and two plants with three fronds were added impartially to each vessel under axenic conditions giving a total number of 10 fronds at test initiation.
Test item analysis:	At test initiation, three days after test beginning, immediately after the renewal and at the end of the test samples of the five highest test concentrations were subjected to analytical determinations of test substance concentrations. The correct application of the lowest test concentration was verified by analysing the stock solutions. The test substance was quantified by reversed phase HPLC. Quantitation was achieved by UV detection of MITC at 248 nm and external calibration using the test substance MITC as reference substance. Prior to the analytical determination, the aqueous MITC samples were acidified with 0.1 % (v/v) hydrochloric acid (10 M)

in order to minimize the possible further hydrolysis of the test substance.

Observations: Frond production and appearance were recorded on days 3, 5 and 7. Every frond visibly projecting beyond the edge of the parent frond was counted. Observations on the appearance of the fronds included necrosis, chlorosis, changes in plant size or shape and root growth.

Statistical evaluation: Means and standard deviations for frond number and growth rates were calculated for each test concentration. The percent inhibition for both parameters relative to the control was determined and the respective concentration response curves drawn. The NOEC was determined using analysis of variance followed by a Dunnett's or William's test. The EC_x was determined using the appropriate model (e.g. probit, logit, log-log or Spearman-Karber). The calculations for this were conducted with a PC and the software package TOXSTAT 3.5 (WEST, Inc.; Western Ecosystems Technology, Inc., 2003 Central Avenue, Cheyenne, WY 82001, USA). Maximum Allowable Toxic Concentration (MATC) is calculated as the geometric mean of the LOEC and the NOEC.

Findings:

Analytical results: At test initiation, the recoveries for MITC were in a range from 79.1 to 115.5 %. 3 days after test initiation the test substance could not be detected anymore in the tested concentrations, which can be attributed to the known hydrolytic instability of MITC. Immediately after the subsequent renewal of the test medium, recoveries of 100.5 to 177.8 % were found for the test substance, again no test substance could be detected after additional 4 days. The correct application of the lowest test concentration (below lowest calibration standard of 0.02 mg a.s./L on the calibration curve) was verified by the determination of the stock solution at test beginning and renewal; recoveries of 102.1 and 103.8 % were yielded. The following biological results are based on mean measured initial concentrations of MITC, except for the lowest concentration which was based on nominal.

Inhibition of biomass and growth: The duckweed population in the control vessels grew sufficiently well, increasing from 10 fronds per vessel to an average of 155 fronds per vessel in the control after 7 days (corresponding to a 15.5 x multiplication or a doubling time of 1.8 d). During the course of the study at a test concentration of 0.942 mg a.s./L the fronds remained smaller and did not separate properly. Besides single fronds got brown and the plants showed shorter roots. At the highest test concentration plants lost their roots and got isolated fronds. Additionally, during the course of the study the fronds changed their colouring from brighter green to brown. At test end in the highest test concentration all fronds were white. No unusual effects were observed at concentrations < 0.942 mg a.s./L. The ECx for growth rate and biomass was determined by log-log analysis.

Table B.2.9.2-100: Measured concentrations of MITC in old and new media at day 0, 3 and 7 in the growth inhibition test with *Lemna gibba*

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)						% of nominal
	Day 0 (new)	Day 3 (3 days old)	Day 3 (new)	Day 7 (4 days old)	% of Day 0	Mean	
control	-	-	-	-	-	-	-
0.01*	-	-	-	-	-	-	-
0.031	0.0248	n.d.[1]	0.0551	n.d. [1]	0	0.0250	62
0.096	0.0759	n.d. [1]	0.1033	n.d. [1]	0	0.0498	31
0.298	0.2380	n.d. [1]	0.3001	n.d. [1]	0	0.1395	17
0.924	0.9562	n.d. [1]	0.9283	n.d. [1]	0	0.4761	11
2.863	3.3057	n.d. [1]	2.9010	n.d. [1]	0	1.5567	6
70.08[2]	71.5302	-	74.4616	-		-	-

[1] for mean calculation half of the lowest calibration sample (0.01 mg/L) was used when substance could not be detected.
[2] MITC stock solution used to prepare the test samples.

Table B.2.9.2-101: Percent inhibition on growth rate and frond number for *Lemna gibba*, exposed to MITC for 7 days under semi-static test conditions

Nominal test concentrations (mg a.s./L)	Mean measured concentrations (mg a.s./L)	% Inhibition after 7 days – growth rate	% Inhibition after 7 days – frond number
negative control	--	--	--
0.01	--*	-0.55	-1.83
0.031	0.040	2.98	8.26
0.096	0.090	3.89	11.01
0.298	0.269	6.73	17.66
0.924	0.942	39.38	70.64
2.863	3.103	91.40	98.17

** Biological results based on nominal concentration for this test concentration as concentration was below the limit of quantification for MITC. Verified by the determination of the stock solution of test beginning and renewal.*

Assessment and conclusions:

The duckweed *Lemna gibba* was exposed for 7 days to a series of six nominal concentrations of MITC ranging from 0.01 to 2.863 mg a.s./L. Frond production and appearance were recorded on days 3, 5 and 7. Observations on the appearance of the fronds included necrosis, chlorosis, changes in plant size or shape and root growth. The test substance degraded rapidly during the course of the test. Therefore water renewal was done 3 days after test initiation. Analytical recoveries at test initiation and after water renewal ranged from 79 % to 178 % of nominal. The following biological results are based on mean measured initial concentrations of MITC, except for the lowest concentration which was based on nominal.

E_rC_{50} (7 day) = 1.18 mg a.s./L (95 % limits: 1.12 - 1.23 mg a.s./L)
E_rC_{10} (7 day) = 0.26 mg a.s./L (95 % limits: 0.24 - 0.29 mg a.s./L)
E_bC_{50} (7 day) = 0.59 mg a.s./L (95 % limits: 0.56 - 0.62 mg a.s./L)
E_bC_{10} (7 day) = 0.10 mg a.s./L (95 % limits: 0.09 - 0.11 mg a.s./L)
NOEC = 0.09 mg a.s./L, LOEC = 0.269 mg a.s./L, MATC = 0.156 mg a.s./L

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.

Endpoints:
E_rC_{50} (*Lemna gibba*, 7 d) = 1.18 mg MITC/L (mean measured)
E_rC_{10} (*Lemna gibba*, 7 d) = 0.26 mg MITC/L (mean measured)
E_bC_{50} (*Lemna gibba*, 7 d) = 0.59 mg MITC/L (mean measured)
E_bC_{10} (*Lemna gibba*, 7 d) = 0.10 mg MITC/L (mean measured)
Analytical method:
Method validation not fully acceptable according to the guidance SANCO/3029/99 rev. 4 on analytical validation but is accepted as fit for purpose. This study is relied upon in the risk assessment.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 221 were met:
- the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 221 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could not be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore it is uncertain to consider it "fit for purpose" The endpoint should be considered with precaution but is not critical.
(please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/61, for further details).
E_rC_{50} (*Lemna gibba*, 7 d, semi-static) = 1.18 mg MITC/L (mean measured)
E_rC_{10} (*Lemna gibba*, 7 d, semi-static) = 0.26 mg MITC/L (mean measured)
E_bC_{50} (*Lemna gibba*, 7 d, semi-static) = 0.59 mg MITC/L (mean measured)
E_bC_{10} (*Lemna gibba*, 7 d, semi-static) = 0.10 mg MITC/L (mean measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic plants.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.7/02.

Data point:	KCA 8.2.7/02
Report author:	[redacted]
Report year:	2019d
Report title:	Effect of Methyl isothiocyanate (Metabolite of BAS 002 N, Dazomet) on the Growth of *Lemna gibba* – Statistical Re-analysis.
Report No.:	CEA.2036
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Guidelines for Testing of Chemicals, No. 221:"*Lemna* sp., Growth Inhibition Test", adopted, 2006 OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006 OECD Guidelines for Testing of Chemicals, No. 210:"Fish Early Life Stage Toxicity Test", adopted July 26 (Annex 6), 2013

Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from BASF Aktiengesellschaft Study code 121437 ([redacted], 2002; KCA 8.2.7/01) for the toxicity of Methyl isothiocyanate (Metabolite of BAS 002 N, Dazomet) to the growth of the freshwater aquatic plant *Lemna gibba* G3 did not provide estimates of the EC_{20} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

The test used nominal concentrations of Methyl isothiocyanate at 0.01, 0.031, 0.096, 0.298, 0.924 and 2.863 mg/L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 79 % to 178 % of the nominal values. In line with the original report, these analyses are based on the mean initial concentrations, with the exception of the lowest concentration, which was based on nominal. The Concentrations used in these analyses were 0.01, 0.04, 0.09, 0.269, 0.942 and 3.103 mg/L.

The following parameters were analysed statistically:

- Yield (Frond Number)
- Growth Rate (Frond Number)
- Biomass (Frond Number)

Statistical analyses of the available data for **yield** revealed that the following E_yC_{10}, E_yC_{20} and E_yC_{50} values were reliably calculated:

Table B.2.9.2-102: Summary of effects on yield for *Lemna gibba*, exposed to MITC for 7 days under semi-static test conditions, statistical re-analysis

Parameter	3 Days			5 Days			7 Days		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_yC_{10}	E_yC_{20}	E_yC_{50}
Value [mg/L]	0.205	0.323	0.768	0.128	0.199	0.466	0.172	0.260	0.576
lower 95 %-cl	0.131	0.232	0.644	0.083	0.145	0.389	0.107	0.183	0.475
upper 95 %-cl	0.276	0.406	0.916	0.171	0.250	0.559	0.233	0.330	0.697

cl: confidence limits

Statistical analyses of the available data for **growth rate** revealed that the following E_rC_{10}, E_rC_{20} and E_rC_{50} values were reliably calculated:

Table B.2.9.2-103: Summary of effects on growth rate for *Lemna gibba*, exposed to MITC for 7 days under semi-static test conditions, statistical re-analysis

Parameter	3 Days			5 Days			7 Days		
	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}
Value [mg/L]	0.281	0.445	1.075	0.204	0.324	0.787	0.411	0.582	1.133
lower 95 %-cl	0.196	0.342	0.933	0.150	0.258	0.692	0.344	0.512	1.056
upper 95 %-cl	0.361	0.539	1.238	0.257	0.387	0.895	0.471	0.645	1.218

cl: confidence limits

Statistical analyses of the available data for **biomass** revealed that the following EC_{10}, EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2-104: Summary of effects on biomass for *Lemna gibba*, exposed to MITC for 7 days under semi-static test conditions, statistical re-analysis

Parameter	3 Days			5 Days			7 Days		
	EC_{10}	EC_{20}	EC_{50}	EC_{10}	EC_{20}	EC_{50}	EC_{10}	EC_{20}	EC_{50}
Value [mg/L]	0.205	0.323	0.768	0.160	0.249	0.582	0.160	0.245	0.556
lower 95%-cl	0.131	0.232	0.644	0.128	0.211	0.528	0.121	0.199	0.492
upper 95%-cl	0.276	0.406	0.916	0.190	0.285	0.643	0.198	0.289	0.630

cl: confidence limits

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Statistical recalculation of endpoints for the study KCA 8.2.7/01 ([redacted] 2002).

Original Endpoints:

E_rC_{50} (*Lemna gibba*, 7 d) = 1.18 mg MITC/L (mean measured)

E_rC_{10} (*Lemna gibba*, 7 d) = 0.26 mg MITC/L (mean measured)

E_bC_{50} (*Lemna gibba*, 7 d) = 0.59 mg MITC/L (mean measured)

E_bC_{10} (*Lemna gibba*, 7 d) = 0.10 mg MITC/L (mean measured)

Endpoints from re-analysis:

E_rC_{50} (*Lemna gibba*, 7 d) = 1.133 mg MITC/L (mean measured)

E_rC_{10} (*Lemna gibba*, 7 d) = 0.411 mg MITC/L (mean measured)

E_bC_{50} (*Lemna gibba*, 7 d) = 0.556 mg MITC/L (mean measured)

E_bC_{10} (*Lemna gibba*, 7 d) = 0.160 mg MITC/L (mean measured)

Assessment and conclusion by Lainco:

The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:

The study is acceptable.

Statistical recalculation of endpoints for the study KCA 8.2.7/01 ([redacted] 2002).

Original Endpoints:

E_rC_{50} (*Lemna gibba*, 7 d) = 1.18 mg MITC/L (mean measured)

E_rC_{10} (*Lemna gibba*, 7 d) = 0.26 mg MITC/L (mean measured)

E_bC_{50} (*Lemna gibba*, 7 d) = 0.59 mg MITC/L (mean measured)

E_bC_{10} (*Lemna gibba*, 7 d) = 0.10 mg MITC/L (mean measured)

Endpoints from re-analysis:

E_rC_{50} (*Lemna gibba*, 7 d) = 1.133 mg MITC/L (mean measured)

E_rC_{10} (*Lemna gibba*, 7 d) = 0.411 mg MITC/L (mean measured)

E_bC_{50} (*Lemna gibba*, 7 d) = 0.556 mg MITC/L (mean measured)
E_bC_{10} (*Lemna gibba*, 7 d) = 0.160 mg MITC/L (mean measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic plants.

Data point:	KCA 8.2.7/03
Report author:	[redacted]
Report year:	2019c
Report title:	Methyl isothiocyanate (MITC) – Effect on the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test under Flow-Through Conditions.
Report No.:	20180077
Document No.:	-
Guidelines followed in study:	OECD 221 (2006) Method C.26 of Commission Regulation (EU) No. 2016/266
Deviations from current test guideline:	Deviations from current OECD guideline 221 (2006): None
Previous evaluation:	No, not previously submitted at EU level
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Lainco S.A. (original Sponsor: Kanesho Soil Treatment, letter of access by Lainco is included, study may be used by Lainco in Europe only)

Study Summary:

The impact of the test item Methyl isothiocyanate (MITC) on the growth of the freshwater aquatic plant *Lemna gibba* (duckweed) was investigated in a 7-day test, based on the OECD Guideline No. 221 "*Lemna* sp. Growth Inhibition Test" (2006) and the Commission Regulation (EC) No. 2016/266, C26.
In this flow-through test, *Lemna gibba* was exposed to five different test item concentrations over a period of 7 days. Toxic effects of the test item on the plant growth based on frond numbers and dry weight, in relation to the solvent control cultures, were evaluated.
The nominal test concentrations tested were 0.016, 0.050, 0.16, 0.50 and 1.6 mg/L. Additionally a control and a solvent control group were tested in parallel. The test concentrations selected were based on a flow-through range-finding test.
The concentrations of Methyl isothiocyanate (MITC) were analytically determined for all test concentrations and the solvent control.
In the samples from the analysed test media the mean measured concentrations (means of 4 sampling dates) of Methyl isothiocyanate (MITC) over the 7-days test period were in the range of 80.1 to 92.0 % of the nominal values. The analytical results showed that under the flow-through conditions the applied concentrations of the test item Methyl isothiocyanate (MITC) could be maintained sufficiently constant during the test period of 7 days. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test concentration.
The biological results are presented based on nominal test item concentrations and on mean measured test item concentrations:

E_rC_{50} (7 day) frond numbers = 0.54 mg MITC/L (95 % confidence interval: 0.52 – 0.56) (nominal)
E_rC_{20} (7 day) frond numbers = 0.30 mg MITC/L (95 % confidence interval: 0.28 – 0.32) (nominal)
E_rC_{10} (7 day) frond numbers = 0.22 mg MITC/L (95 % confidence interval: 0.20 – 0.25) (nominal)

E_yC_{50} (7 day) frond numbers = 0.28 mg MITC/L (95 % confidence interval: 0.26 – 0.30) (nominal)
E_yC_{20} (7 day) frond numbers = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.14) (nominal)
E_yC_{10} (7 day) frond numbers = 0.083 mg MITC/L (95 % confidence interval: 0.069 – 0.096) (nominal)

E_rC_{50} (7 day) dry weight of the plants (biomass) = 0.35 mg MITC/L (95 % confidence interval: 0.33 – 0.37) (nominal)
E_rC_{20} (7 day) dry weight of the plants (biomass) = 0.19 mg MITC/L (95 % confidence interval: 0.17 – 0.21) (nominal)
E_rC_{10} (7 day) dry weight of the plants (biomass) = 0.14 mg MITC/L (95 % confidence interval: 0.12 – 0.16) (nominal)
E_yC_{50} (7 day) dry weight of the plants (biomass) = 0.24 mg MITC/L (95 % confidence interval: 0.21 – 0.26) (nominal)
E_yC_{20} (7 day) dry weight of the plants (biomass) = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.15) (nominal)
E_yC_{10} (7 day) dry weight of the plants (biomass) = 0.094 mg MITC/L (95 % confidence interval: 0.074 – 0.11) (nominal)

E_rC_{50} (7 day) frond numbers = 0.43 mg MITC/L (95 % confidence interval: 0.42 – 0.45) (mean measured)
E_rC_{20} (7 day) frond numbers = 0.25 mg MITC/L (95 % confidence interval: 0.23 – 0.26) (mean measured)
E_rC_{10} (7 day) frond numbers = 0.18 mg MITC/L (95 % confidence interval: 0.17 – 0.20) (mean measured)
E_yC_{50} (7 day) frond numbers = 0.24 mg MITC/L (95 % confidence interval: 0.22 – 0.26) (mean measured)
E_yC_{20} (7 day) frond numbers = 0.12 mg MITC/L (95 % confidence interval: 0.10 – 0.13) (mean measured)
E_yC_{10} (7 day) frond numbers = 0.079 mg MITC/L (95 % confidence interval: 0.066 – 0.090) (mean measured)

E_rC_{50} (7 day) dry weight of the plants (biomass) = 0.29 mg MITC/L (95 % confidence interval: 0.28 – 0.31) (mean measured)
E_rC_{20} (7 day) dry weight of the plants (biomass) = 0.17 mg MITC/L (95 % confidence interval: 0.16 – 0.19) (mean measured)
E_rC_{10} (7 day) dry weight of the plants (biomass) = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.14) (mean measured)
E_yC_{50} (7 day) dry weight of the plants (biomass) = 0.20 mg MITC/L (95 % confidence interval: 0.19 – 0.23) (mean measured)
E_yC_{20} (7 day) dry weight of the plants (biomass) = 0.12 mg MITC/L (95 % confidence interval: 0.10 – 0.14) (mean measured)
E_yC_{10} (7 day) dry weight of the plants (biomass) = 0.091 mg MITC/L (95 % confidence interval: 0.073 – 0.11) (mean measured)

In conclusion, the overall NOEC of the test was determined to be 0.050 mg MITC/L nominal (0.046 mg MITC/L mean measured) since the test item did not cause relevant toxic effects on the growth of *Lemna* (frond number and dry weight) during the exposure period of 7 days.
The concentration of 0.16 mg MITC/L nominal (0.14 mg MITC/L mean measured) was determined to be the overall LOEC due to the statistically significantly reduced growth of *Lemna* (frond number and biomass) at this test concentration.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 %
Test species:	Duckweed (*Lemna gibba*)

Type of test:	Flow-through toxicity test (35-fold theoretical test medium exchange rate per day per replicate)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control: 50 µl/L N,N-dimethylformamide), 0.016, 0.050, 0.16, 0.50 and 1.6 mg MITC/L
Dilution medium:	20x AAP medium
Number of organisms, number of replicates:	3 randomly selected colonies per vessel (12 fronds/3 colonies), 3 replicates for the control, 3 replicates for the solvent control and 3 replicates per treatment group
Time of exposure:	7 days
Test conditions:	temperature: 24 °C pH: 7.6 at start, 8.2 at end photoperiod: continuous illumination light intensity: 99 – 107 µE m^{-2} s^{-1}
Test procedure:	Pre-test: 7 day range-finding test under flow-through conditions for the selection of the test concentrations for the main test. Main test: flow-through dose-response test with 3 replicates of randomly selected and aseptically transferred 12 fronds/3 colonies each per concentration; 0.016, 0.050, 0.16, 0.50 and 1.6 mg/L (nominal), control (test water without test item) and solvent control (test water without test item but with addition of solvent). The flow-through test design was chosen to keep the concentrations of the volatile test item as constant as possible during the exposure period. The duration of the test was 7 days, the concentrations were maintained using a computer-controlled dosing system.
Test item analysis:	For the analysis of the test item concentrations in the freshly prepared DMF-application solutions, one sample was taken from all test concentrations from the two application solution preparation dates (Day -1 and Day 3). For the analysis of the test item concentrations in the test media, duplicate samples from the test media of all test concentrations and from the solvent control were taken at the start of the test (Day 0), at Day 3 and Day 5 and at the end of the test at Day 7. The concentration of MITC in the lemna test medium was determined by gas chromatography with mass spectrometric detection (GC-MS) using external calibration including an internal standard.
Observations:	On days 2 and 5 and at the end of the test on day 7, the number of fronds and colonies of the *Lemna gibba* plants were counted. Fronds visibly projecting over the edge of the mother frond were counted as separate fronds. Additionally, the plants were inspected for changes in appearance (e.g. discoloration, sinking, root length, or other abnormalities). The dry weight of a sample of twelve fronds was determined at the start of the test. At the test termination, the dry weight of the plants of each test vessel was determined. The pH and the water temperature was measured and recorded in each treatment at the start of the test and at least at days 3, 5 and 7. Additionally, the temperature was continuously measured and recorded by a temperature recorder in a control test vessel.

	The appearance of the application solutions and the test media in the mixing vessels and test vessels was checked each working day.
Statistical evaluation:	The NOEC and LOEC were determined by testing the parameters at the test concentrations for statistically significant differences to the solvent control values using the Williams t-test. The EC_{10}, EC_{20} and EC_{50} values for the inhibition of the growth rate and yield (based on frond numbers and dry weight) and their 95 % confidence limits were calculated by Probit Analysis using linear maximum likelihood regression. Statistical analysis was performed using ToxRat Professional®.

Findings:

Analytical results:	In the samples from the analysed test media the mean measured concentrations (means of 4 sampling dates) of Methyl isothiocyanate (MITC) over the 7-days test period were in the range of 80 to 92.0 % of the nominal values. The ranges of individual measurements are shown in the table below. The analytical results showed that under the flow-through conditions the applied concentrations of the test item Methyl isothiocyanate (MITC) could be maintained sufficiently constant during the test period of 7 days. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test concentration. The application solutions, the test media in the mixing chambers of the dosing units and the test media in the test vessels appeared to be clear solutions throughout the test period. No remarkable observations (e.g. precipitation or turbidity) were made.
Inhibition of biomass and growth:	The growth of *Lemna gibba* (growth rate and yield based on frond number and biomass) in the control was not statistically different compared to the solvent control. The test item had no statistically significant inhibitory effect on the number of fronds (growth rate) and on the dry weight (growth rate and yield) after the exposure period of 7 days at the concentrations up to and including 0.050 mg/L nominal (0.046 mg/L mean measured). Only the yield based on number of fronds was statistically significantly inhibited first at this test concentration of 0.050 mg/L nominal (0.046 mg/L mean measured) according to the Williams test. The inhibitory effect at this concentration was 11 %. This low inhibition compares to an EC_{10} value, which is generally accepted as a surrogate for the NOEC. Therefore, the NOEC for yield based on number of fronds was determined to be at the test concentration of 0.050 mg/L nominal (0.046 mg/L mean measured), despite the result of the statistical test. This experimental determined NOEC is comparable to the calculated EC_{10} value for yield based on number of fronds which was 0.083 mg/L nominal (0.079 mg/L mean measured). The overall NOEC of the test was determined to be 0.050 mg/L nominal (0.046 mg/L mean measured) as no relevant toxic effect on the growth of *Lemna gibba* (number of fronds and dry weight) was observed up to and including this test concentration.

At 0.16 mg/L nominal (0.14 mg/L mean measured) and all higher test concentrations, the growth of *Lemna gibba*, based on all endpoints assessed, was statistically significant inhibited. The concentration of 0.16 mg/L nominal (0.14 mg/L mean measured) was determined to be the overall LOEC due to the statistically significantly reduced growth of *Lemna gibba* (frond number and biomass) compared to the solvent control.
No abnormalities in appearance of the test plants were recorded in the control, the solvent control and the test concentrations up to and including 0.050 mg/L nominal (0.046 mg/L mean measured). At the concentration of 0.16 mg/L nominal (0.14 mg/L mean measured) a part of the fronds was yellowish coloured, indicating a toxic effect of the test item. At the two highest test concentration discoloration and necrosis of the fronds and shortened root length was observed.

Table B.2.9.2-105: Measured concentrations of MITC in the growth inhibition test with *Lemna gibba*

Nominal concentration of MITC [mg/L]	Range of the individual measurements [mg/L]	Standard deviation	Mean measured concentration of MITC [mg/L]	% of Nominal concentration
0.016	0.0131 – 0.0162	0.001	0.015	92.0
0.050	0.0399 – 0.0518	0.005	0.046	91.3
0.16	0.130 – 0.157	0.011	0.14	89.8
0.50	0.387 – 0.426	0.017	0.40	80.6
1.6	1.25 – 1.30	0.020	1.3	80.1

Table B.2.9.2-106: EC_{10}, EC_{50}, EC_{100}, LOEC and NOEC values (mean measured) of *Lemna gibba*, exposed to MITC for 7 days under flow-through test conditions

	7-Day EC Values [mg/L]			
	Frond Numbers		Dry Weight of the Plants (Biomass)	
	Growth Rate	Yield	Growth Rate	Yield
EC_{10}	0.18	0.079	0.13	0.091
95 % C.I.	0.17-0.20	0.066-0.090	0.11-0.14	0.073-0.11
EC_{20}	0.25	0.12	0.17	0.12
95 % C.I.	0.23-0.26	0.10-0.13	0.16-0.19	0.10-0.14
EC_{50}	0.43	0.24	0.29	0.20
95 % C.I.	0.42-0.45	0.22-0.26	0.28-0.31	0.19-0.23
NOEC	0.046	0.046	0.046	0.046
LOEC	0.14	0.14	0.14	0.14

95 % C.I.: 95 % confidence interval

Assessment and conclusions:

The overall NOEC of the test was determined to be 0.050 mg/L nominal (0.046 mg/L mean measured) since the test item did not cause relevant toxic effects on the growth of *Lemna gibba* (frond number and dry weight) during the exposure period of 7 days.
The concentration of 0.16 mg/L nominal (0.14 mg/L mean measured) was determined to be the overall LOEC due to the statistically significantly reduced growth of *Lemna gibba* (frond number and biomass) compared to the solvent control.

E_rC_{50} (7 day) frond numbers = 0.43 mg MITC/L (95 % confidence interval: 0.42 – 0.45) (mean measured)
E_rC_{20} (7 day) frond numbers = 0.25 mg MITC/L (95 % confidence interval: 0.23 – 0.26) (mean measured)

E_rC_{10} (7 day) frond numbers = 0.18 mg MITC/L (95 % confidence interval: 0.17 – 0.20) (mean measured)
E_yC_{50} (7 day) frond numbers = 0.24 mg MITC/L (95 % confidence interval: 0.22 – 0.26) (mean measured)
E_yC_{20} (7 day) frond numbers = 0.12 mg MITC/L (95 % confidence interval: 0.10 – 0.13) (mean measured)
E_yC_{10} (7 day) frond numbers = 0.079 mg MITC/L (95 % confidence interval: 0.066 – 0.090) (mean measured)

E_rC_{50} (7 day) dry weight of the plants (biomass) = 0.29 mg MITC/L (95 % confidence interval: 0.28 – 0.31) (mean measured)
E_rC_{20} (7 day) dry weight of the plants (biomass) = 0.17 mg MITC/L (95 % confidence interval: 0.16 – 0.19) (mean measured)
E_rC_{10} (7 day) dry weight of the plants (biomass) = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.14) (mean measured)
E_yC_{50} (7 day) dry weight of the plants (biomass) = 0.20 mg MITC/L (95 % confidence interval: 0.19 – 0.23) (mean measured)
E_yC_{20} (7 day) dry weight of the plants (biomass) = 0.12 mg MITC/L (95 % confidence interval: 0.10 – 0.14) (mean measured)
E_yC_{10} (7 day) dry weight of the plants (biomass) = 0.091 mg MITC/L (95 % confidence interval: 0.073 – 0.11) (mean measured)

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is not mentioned in the dossier of the applicant Taminco.
Assessment and conclusion by Lainco:
The study is acceptable.
E_rC_{50} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.43 mg MITC/L (95 % confidence interval: 0.42 – 0.45) (mean measured)
E_rC_{20} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.25 mg MITC/L (95 % confidence interval: 0.23 – 0.26) (mean measured)
E_rC_{10} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.18 mg MITC/L (95 % confidence interval: 0.17 – 0.20) (mean measured)
E_rC_{50} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.29 mg MITC/L (95 % confidence interval: 0.28 – 0.31) (mean measured)
E_rC_{20} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.17 mg MITC/L (95 % confidence interval: 0.16 – 0.19) (mean measured)
E_rC_{10} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.14) (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 221 were met:
- the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1} (measured: 1.8 days)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 221 when reporting the data.
Therefore, this study is still considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered “fit for purpose” (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/23, for further details).

E_rC_{50} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.43 mg MITC/L (95 % confidence interval: 0.42 – 0.45) (mean measured)
E_rC_{20} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.25 mg MITC/L (95 % confidence interval: 0.23 – 0.26) (mean measured)
E_rC_{10} (*Lemna gibba*, 7 day, flow-through) frond numbers = 0.18 mg MITC/L (95 % confidence interval: 0.17 – 0.20) (mean measured)
E_rC_{50} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.29 mg MITC/L (95 % confidence interval: 0.28 – 0.31) (mean measured)
E_rC_{20} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.17 mg MITC/L (95 % confidence interval: 0.16 – 0.19) (mean measured)
E_rC_{10} (*Lemna gibba*, 7 day, flow-through) dry weight = 0.13 mg MITC/L (95 % confidence interval: 0.11 – 0.14) (mean measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic plants.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No studies submitted.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

The relevant studies on the chronic aquatic toxicity of metam are shown in Table 2.9.2.2-1 above (algae and aquatic plants). The relevant studies on the chronic aquatic toxicity of MITC are shown in Table 2.9.2.2-3. These table contain all information currently available in the dossier submitted for the current Annex I renewal application of metam.

Table 2.9.2.2-3 Summary of relevant information on chronic aquatic toxicity for MITC

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
Fish						
Chronic fish prolonged toxicity study based on OECD 204 GLP	*Oncorhynchus mykiss*	MITC, Purity: 98.4%, Batch no.: CP 4011	NOEC = 0.004 mg MITC/L (mean measured) EC_{10} = 0.017 mg MITC/L (nominal)	Not acceptable for risk assessment and classification	28 d flow-through 20 fish/ replicate 1 replicate/ treatment	CA8.2.2/01 [redacted], 1990 CA8.2.2/02 [redacted], 2019b
Chronic fish early life stage toxicity study based on OECD 210 GLP	*Pimephales promelas*	MITC, Purity: 97.2 %, Batch no.: 56198PJV	NOEC = 0.00774 mg MITC/L EC_{10} = 0.00924 mg MITC/L (mean measured)	Acceptable Key study	33 d flow-through 20 embryos/ replicate 4 replicates/ control 4 replicates/ treatment	CA8.2.2.1/01 [redacted], 2015
Aquatic invertebrates						
Chronic Daphnia reproductive toxicity study based on OECD 202 Part II, EEC XI/691/86, draft 4, DIN 38 412 (draft, 1981), US EPA 660/3-75-009	*Daphnia magna*	MITC, Purity: not reported, Batch no.: 340401/1	NOEC = 0.00625 mg MITC/L (nominal)	Endpoint is uncertain Not acceptable for classification	21 d semi-static 1 daphnid/ replicate 10 replicates/ treatment	CA8.2.5.1/01 [redacted], 2001
Chronic daphnia reproductive toxicity study based on OECD 211, EC 440/2008 Method C20	*Daphnia magna*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	NOEC = 0.0211 mg MITC/L EC_{10} = 0.035 mg MITC/L (mean measured)	Acceptable Key study	21 d flow-through 1 daphnid/ replicate 10 replicates/ treatment	CA8.2.5.1/03 [redacted]., 2019c
Algae						
Algal growth inhibition study based	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.0 %, Batch no.: 80525	E_bC_{50} = 0.281 mg MITC/L E_rC_{50} =	Acceptable Supportive study	72 h static Initial cell count: 1 x 10^4 cells/mL	CA8.2.6.1/05 [redacted] 1998

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
on OECD 201 GLP			0.432 mg MITC/L (initial measured)		5 replicates/ control 10 replicates/ solvent control 5 replicates/ treatment	CA8.2.6.1/06 [redacted], 2019a
Algal growth inhibition study based on OECD 201, EU 92/69/EECC Method C3, US EPA OPPTS 850.5400, ISO 14442 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_yC_{50} = 0.12 mg MITC/L E_yC_{10} = 0.08 mg MITC/L E_rC_{50} = 0.21 mg MITC/L E_rC_{10} = 0.09 mg MITC/L $NOAEC_y$ = 0.044 mg MITC/L $NOAEC_r$ = 0.11 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/07 [redacted], 2012d
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} = 0.189 mg MITC/L E_rC_{10} = 0.076 mg MITC/L E_yC_{50} = 0.091 mg MITC/L E_yC_{10} = 0.051 mg MITC/L E_bC_{50} = 0.0933 mg MITC/L E_bC_{10} = 0.0456 mg MITC/L (mean measured)	Acceptable Key study	72 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/09 [redacted], 2018b CA8.2.6.1/10 [redacted], 2020d
Algal growth inhibition	*Anabaena flos-aquae*	MITC, Purity: 99.6 %, Batch no.: 408208/1	E_rC_{50} = 3.607 mg MITC/L	Acceptable Supportive	72 h static Initial cell count: 3 x	CA8.2.6.2/01 [redacted], 2002

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
study based on ASTM E 1218-90, OECD 201, OPPTS 850.1000 GLP			E_rC_{10} = 1.143 mg MITC/L E_yC_{50} = 1.886 mg MITC/L E_yC_{10} = 0.793 mg MITC/L (initial measured)	study	10^4 cells/mL 10 replicates/ control 5 replicates/ treatment	CA8.2.6.2/02 [redacted], 2019b
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPPTS 850.5400, ISO 14442 GLP	*Anabaena flos-aquae*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_rC_{50} (72 h) = 0.433 mg MITC/L E_yC_{50} (72 h) = 0.431 mg MITC/L E_bC_{50} (96 h) = 0.1792 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/03 [redacted], 2012a CA8.2.6.2/04 [redacted] 2020
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPTS 850.5400, ISO 14442 GLP	*Navicula pelliculosa*	MITC, Purity: 99.7 %, Batch no.:56198PJV	E_rC_{50} (72 h) = 0.349 mg MITC/L E_rC_{10} (72 h) = 0.144 mg MITC/L E_yC_{50} (72 h) = 0.181 mg MITC/L E_bC_{50} (72 h) = 0.1804 mg MITC/L (initial measured)	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 8 replicates/ control 4 replicates/ treatment	CA8.2.6.2/05 [redacted], 2012b CA8.2.6.2/06 [redacted], 2020a
Algal growth inhibition study based on OECD 201, EU 92/69/EEC Method C3, US EPA OPPTS	*Skeletonema costatum*	MITC, Purity: 99.7 %, Batch no.: 56198PJV	E_rC_{50} (72 h) > 0.430 mg MITC/L E_rC_{10} (72 h) = 0.0351 mg MITC/L E_yC_{50} (72	Acceptable Supportive study	96 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/07 [redacted], 2012c CA8.2.6.2/08 [redacted], 2020b

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
850.5400, ISO 14442 GLP			h) = 0.081 mg MITC/L E_yC_{10} (72 h) = 0.0246 mg MITC/L E_bC_{50} (72 h) = 0.1391 mg MITC/L E_bC_{10} (72 h) = 0.021 mg MITC/L (initial measured)			
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3	*Anabaena flos-aquae*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (72 h) = 0.375 mg MITC/L E_rC_{10} (72 h) = 0.173 mg MITC/L E_yC_{50} (72 h) = 0.181 mg MITC/L E_yC_{10} (72 h) = 0.051 mg MITC/L E_bC_{50} (72 h) = 0.208 mg MITC/L E_bC_{10} (72 h) = 0.067 mg MITC/L (nominal)	Acceptable Supportive study	72 h static Initial cell count: 10000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.2/09 [redacted], 2019 CA8.2.6.2/10 [redacted], 2020e
Lemna growth inhibition study based on OECD 221, OPPTS 850.5400, ASTM E1415-91, EPA J, 132-2	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: 408208/1	E_rC_{50} = 1.133 mg MITC/L E_rC_{10} = 0.411 mg MITC/L E_bC_{50} = 0.556 mg MITC/L E_bC_{10} =	Endpoint is uncertain Not acceptable for classification	7 d semi-static Inoculation with one plant with 4 fronds and two plants with 3 fronds, 6 replicates/ control	CA8.2.7/01 [redacted], 2002 CA8.2.7/02 [redacted], 2019d

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
			0.160 mg MITC/L (mean measured)		3 replicates/ treatment	
Lemna growth inhibition study based on OECD 221, EC 2016/266 Method C26	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (frond numbers) = 0.43 mg MITC/L E_rC_{10} (frond numbers) = 0.18 mg MITC/L E_rC_{50} (dry weight) = 0.29 mg MITC/L E_rC_{10} (dry weight) = 0.13 mg MITC/L (mean measured)	Acceptable Key study	7 d flow-through Inoculation with 3 randomly selected colonies per vessel (12 fronds/ 3 colonies) 3 replicates/ control 3 replicates/ solvent control 3 replicates/ treatment	CA8.2.7/03 [redacted], 2019c

2.9.2.3.1 Chronic toxicity to fish

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. 2 chronic toxicity studies with fish are available for MITC.

Studies with MITC

Data point:	KCA 8.2.2/01
Report author:	[redacted]
Report year:	1990
Report title:	Sublethal toxic effects on rainbow trout (*Salmo gairdneri* RICH. = *Oncorhynchus mykiss*) of Methylisothiocyanate (MITC)
Report No.:	-
Document No.:	[redacted]
Guidelines followed in study:	OECD 204, 1984
Deviations from current test guideline:	Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	This study type is no longer required according to the EU data requirements. As this study is available it is presented in this renewal dossier. However, this study is not relied upon in risk assessment.

Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF Aktiengesellschaft)

Study Summary:

Methylisothiocyanate (MITC) was tested for its sublethal toxic effects on Rainbow trout (*Oncorhynchus mykiss*), 20 fish/test group, in the concentrations 0 (control), 0 (solvent control), 0.001, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg/L in a flow-through system for a period of 4 weeks. Actual measured concentrations of MITC after 4 weeks were between 60 – 66 %.
Compound-related mortalities occurred in the test groups 0.02 mg/L (25 %), 0.05 mg/L (60 %), 0.1 and 0.2 mg/L (each 100 %) increasing with increase in the concentration and duration of the exposure. In the control and the solvent control groups as well as in the lower concentrations (0.001 to 0.01 mg/L) no mortalities occurred. Compound-related toxic signs observed were: reduced or no feed consumption, discoloration (dark), apathy, lying on the bottom, swimming near the bottom, spasms and convulsions and narcosis-like state. Compared with the control group the mean body weight and length in the three highest test groups with surviving fish (0.01, 0.02 and 0.05 mg/L) were statistically significantly smaller at the end of the study.
Under the conditions of this study the "No Observed Effect Concentration" (= NOEC) was determined to be 0.005 mg/L (nominal concentration) for the Rainbow trout (*Oncorhynchus mykiss*). The threshold level for lethal effects was greater than 0.01 and less than 0.02 mg/L (nominal concentrations). The threshold level for toxicity (= symptoms) as well as for the deve1opment of body weight and length was greater than 0.005 and less than 0.01 mg/L (nominal concentrations).

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: CP 4011, chemical purity: 98.4 %
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Age, weight, length, loading:	Age: juveniles, approximately 8 months old, mean weight at start: 7.9 g (5.6 – 10.4 g), mean length at start: 8.9 cm (7.2 – 10.2 cm), fish loading: 0.22 g fish/L/day
Acclimatisation of the fish:	8 weeks acclimatisation
Medical treatment:	Soon after arrival in the laboratory the fish were treated twice with 0.05 mg/L malachite green chloride and once with 10 mg/L tetracycline hydrochloride for about 24 hours each.
Feeding:	"Ssniff" starter trout diet - During acclimatisation: about 2 % of their mean body weight daily in 2 portions on workdays and in 1 portion on non-working days - During study: about 4 % of their mean body weight on workdays daily in 2 portions and on non-working days in 1 portion. Beginning with week 2 of the study the amount of feed was generally increased by 25 % each week (calculated for the initial weight in each case and the number of survivors if applicable)
Type of test:	Flow-through toxicity test (7.2 volume replacements per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control: acetone), 0.001, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg MITC/L
Number of animals per group:	20 fish for the control, for the solvent control and per treatment group

Time of exposure:	28 days In-life dates: November 14th to December 12th 1989
Test conditions:	temperature: 15 °C dissolved oxygen: 7.7 – 10.9 mg/L O_2, 75 – 107 % O_2 saturation pH: 7.5 – 7.9 total hardness: 2.3 – 2.4 mmol/L alkalinity: not reported photoperiod: 16 hours light and 8 hours dark light intensity: not reported
Test procedure:	The Rainbow trout (*Oncorhynchus mykiss*) were exposed by groups of 20 for 4 weeks to nominal concentrations: 0 (control), 0 (solvent control), 0.001, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg test item/L in an flow-through system. The stock solutions with concentrations ranging from 0.3 to 60 mg/L were prepared with acetone. From these stock solutions in acetone aqueous stock solutions were prepared once weekly. Definite quantities of these aqueous solutions were admixed to definite quantities of bidistilled water. The stock solutions in the storage vessels were replaced once a week. The flow rates of the test solutions in the test aquaria were 30 L/h.
Test item analysis:	An analytical method for the determination of the test compound in the test water was performed by HPLC and it was conducted at the responsible unit "BASF Laboratory for Analytical and Preparative chemistry", BASF Agricultural Research Station, D-6703 Limburgerhof, FRG. The homogeneity was guaranteed since the test compound was completely dissolved in water. Samples of the test water were taken at about weekly intervals from all test aquaria except from the lowest concentration 0.001 mg/L and analyzed for concentration of the test compound. Since the detection limit of the analytical method was 0.005 mg/L, for the lowest test concentration 0.001 mg/L the diluted stock solution with a concentration of 0.3 mg/L was analyzed instead. Prior to the beginning of the study the stability of the diluted stock solutions was verified for a period of 7 days for the stock solutions containing 0.3 (group 2), 3 (group 4) and 15 mg/L (group 6). The samples for the weekly concentration controls during the study had generally always been taken out of the aquaria immediately before the diluted stock solutions in the storage vessels were replaced except at the beginning of the study.
Observations:	Mortality was determined daily. Clinical signs were assessed at least on each workday. The individual body weights and body lengths (from the tip of the mouth to the distal end of the caudal fin) were determined at the beginning (day 0) and end (day 28) of the study. About 12 hours before the determination of the body weight the feed was withdrawn. Temperature was measured daily, oxygen content and pH were measured twice weekly.
Statistical evaluation:	The statistical evaluation of the body weight and body length at the beginning (day 0) and at the end (28 day) of the study was performed by one-way analysis (ANOVA) followed by Dunnett`s test: A multiple comparison procedure for comparing several treatments with a control (Dunnett, 1955).

Findings:

Analytical results: At the start of the test, concentration values in the range of 87 – 107 % for recovery were determined. At the end of the test, the analytically determined values were in a range of 60 - 66 %. However, values presented in the result section are given in nominal content.

Mortality, behaviour and clinical signs: Compound-related mortalities occurred in the test groups 0.02 mg/L (25 %), 0.05 mg/L (60 %), 0.1 and 0.2 mg/L (each 100 %) increasing with increase in the concentration and duration of the exposure. In the control and the solvent control groups as well as in the lower concentrations (0.001 to 0.01 mg/L) no mortalities occurred.

The lowest concentration at which mortalities occurred was 0.02 mg/L. The threshold level of lethal effects was greater than 0.01 and smaller than 0.02 mg/L. The highest concentration tested without mortalities was 0.01 mg/L.

Compound-related toxic signs were observed from the concentration 0.01 mg/L upward, increasing with increase in the concentration and duration of exposure.

Compound-related toxic signs observed were: reduced or no feed consumption, discoloration (dark), apathy, lying on the bottom, swimming near the bottom, spasms and convulsions and narcosis-like state.

Body weight and body length: Compared with the control group the mean body weight and length in the three highest test groups with surviving fish (0.01, 0.02 and 0.05 mg/L) were statistically significantly smaller (p = 0.01) at the end of the study. Under the conditions of this study, the no effect concentration for the growth parameters body weight and length gain was thus 0.005 mg/L.

Table B.2.9.2-107: Results of the analytical concentration control (mean of two determinations)

Nominal concentration (mg/L)	Measured concentration (mg/L)							% of nominal concen-tration
	Day 0	Day 7	Day 14	Day 21	Day 28	% of day 0	Mean	
control	0	0	0	0	n.d.	-		
solvent control	0	0	0	0	n.d.	-		
0.3*	0.250	0.288	0.291	0.207	0.197	78.8%	0.247	82.2%
0.005	n.d.[2]	n.d.[2]	n.d.[2]	n.d.[2]	n.d.[2]	n.c.	0.0025[2]	50.0%
0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.c.	n.c.	-
0.02	0.018	0.021	n.i.	n.i.	n.i.	n.c.	0.0195	97.5%
0.05	0.054	n.d.[2]	0.028	0.048	0.030	55.5%	0.0325[2]	65.0%
0.1[1]	-	-	-	-	-	-	-	-
0.2[1]	-	-	-	-	-	-	-	-

[1] Added concentration (day 7) not analyzed

[2] for mean calculation half of the limit of the analytical method (0.0025 mg/L) was used when concentrations were not detectable

** The stock solution (0.3 mg/L) was analysed instead of the concentration in the aquarium (0.001 mg/L)*

n.d. = not detectable; n.i. = non-integrable due to peak overlap); n.c. = not calculable

Table B.2.9.2-108: Cumulative mortality for Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 28 days under flow-through test conditions

Nominal concentration (mg/L)	Measured concentration 28 days (mg/L)	Number of fish	Cumulative mortality (number of dead organisms)				
			1 day	7 days	14 days	21 days	28 days
control	0	20	0	0	0	0	0
solvent control	0	20	0	0	0	0	0
0.001*	0.197*	20	0	0	0	0	0
0.005	n.d.	20	0	0	0	0	0
0.01	n.i.	20	0	0	0	0	0
0.02	n.i.	20	0	0	1	2	5
0.05	0.030	20	0	0	2	7	12
0.1	-	20	-	0	19	-	-
0.2	-	20	-	0	-	-	-

** The stock solution (0.3 mg/L) was analysed instead of the concentration in the aquarium (0.001 mg/L)*
n.d. = not detectable; n.i. = non-integrable due to peak overlap

Table B.2.9.2-109: Symptoms for Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 28 days under flow-through test conditions

Nominal concentration (mg/L)	Measured concentration 28 days (mg/L)	Number of fish	Symptoms				
			1 day	7 days	14 days	21 days	28 days
control	0	20	n.a.	n.a.	n.a.	n.a.	n.a.
solvent control	0	20	n.a.	n.a.	n.a.	n.a.	n.a.
0.001	0.197*	20	n.a.	n.a.	n.a.	n.a.	n.a.
0.005	n.d.	20	n.a.	n.a.	n.a.	n.a.	n.a.
0.01	n.i.	20	n.a.	n.a.	K4/20, P1/20, V	K2/20, P2/20, V, Z	K3/20, P3/20, V
0.02	n.i.	20	n.a.	K1/20	K5/19, Bo, V, Z	A, Bo, K, P6/18, V, Z	A, Bo, K4/15, P4/15, V
0.05	0.030	20	n.a.	Bo, V, Z	Bo, K, N1/18, V, Z	Bo, K1/13 P1/13, V, Z	A, Bo, K1/8, P1/8, V
0.1	-	20	-	o.B.	Bo, K, V, Z	-	-
0.2	-	20	-	o.B.	-	-	-

** The stock solution (0.3 mg/L) was analysed instead of the concentration in the aquarium (0.001 mg/L)*
n.d. = not detectable; n.i. = non-integrable due to peak overlap
A = apathy; B = distended abdomen; Bo = swimming near the bottom; E = exophthalmos; F = attempts to escape; G = aggressiveness; H = hyperreflexia; K = spasms, convulsions; L = gasping, N = narcosis-like state; O = swimming at the surface; P = lying on the bottom; S = side position; T = tumbling; U = restlessness; V = discoloration (dark); W = headstand; X = accelerated respiration; Z = reduced (no) feed consumption; n.a. = no abnormalities; - = not assessed.
Numbers after letters: e.g. 2/9 = 2 out of 9 fish still alive affected; if no number behind letter, number not safely determinable ( ) = slight to very slight.

Table B.2.9.2-110: Body weight and body length for Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 28 days under flow-through test conditions

Nominal concentration (mg/L)	Measured concentration 28 days (mg/L)	Number of fish	Mean body weight ± SD (g)		Mean body length ± SD (cm)	
			Beginning of study	End of study	Beginning of study	End of study
control	0	20	7.69 ± 1.079	19.94 ± 3.867	8.93 ± 0.447	12.04 ± 0.685
solvent control	0	20	8.20 ± 0.981	21.50 ± 3.190	9.17 ± 0.367	12.25 ± 0.619
0.001	0.197*	20	7.91 ± 1.121	21.18 ± 3.817	9.02 ± 0.473	12.20 ± 0.825
0.005	n.d.	20	7.94 ± 1.064	17.86 ± 4.532	9.09 ± 0.482	11.73 ± 0.994
0.01	n.i.	20	8.18 ± 1.174	14.39 ± 5.115	9.10 ± 0.498	10.57 ± 1.100
0.02	n.i.	20	8.11 ± 1.102	10.28 ± 1.952	9.09 ± 0.425	9.41 ± 0.414
0.05	0.030	20	7.64 ± 1.118	9.10 ± 0.760	9.01 ± 0.480	9.39 ± 0.416
0.1	-	20	7.49 ± 1.681	-	8.58 ± 0.806	-
0.2	-	20	7.57 ± 1.737	-	8.53 ± 0.915	-

** The stock solution of 0.3 mg/L was analysed instead of the concentration in the aquarium (0.001 mg/L)*
SD = Standard deviation; n.d. = not detectable; n.i. = non-integrable due to peak overlap

Assessment and conclusions:

Under the conditions of this study the "No Observed Effect Concentration" (= NOEC) was determined to be 0.005 mg/L (nominal concentration) for the Rainbow trout (*Oncorhynchus mykiss*). The threshold level for lethal effects was greater than 0.01 and less than 0.02 mg/L (nominal concentrations). The threshold

level for toxicity (= symptoms) and for the deve1opment of body weight and length was greater than 0.005 and less than 0.01 mg/L (nominal concentrations).

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable as supplementary data.
Endpoints:
NOEC (*Oncorhynchus mykiss*, 28 d) = 0.005 mg MITC/L (nominal)
NOEC (*Oncorhynchus mykiss*, 28 d) = 0.004 mg MITC/L (mean measured)
According to PRAPeR 53 (August 2008), for the recalculation of the endpoint in mean measured concentrations, the mean recovery of the concentration 0.05 mg MITC/L of 80 % was used.
The NOEC is based on effects on growth (body weight and body length) and clinical signs.
Analytical method:
Despite some minor deviations from the guidance SANCO/3029/99 rev. 4 on analytical validation, the method is assessed to be fit for purpose.
Assessment and conclusion by Lainco:
The study is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
Endpoints:
NOEC (*Oncorhynchus mykiss*, 28 d, flow-through) = 0.005 mg MITC/L (nominal)
NOEC (*Oncorhynchus mykiss*, 28 d, flow-through) = 0.004 mg MITC/L (mean measured)
The analytical method used could not be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and it cannot be considered “fit for purpose” (please refer to Vol. 3 (CA), Section B.5..1.2.6 – KCA 4.1.2/49, for further details).
Since the OECD Guideline 204 is no longer supported in current aquatic risk assessment, the fish juvenile growth study is considered acceptable as supplementary data and therefore not relied upon.
An alternative fish early life stage toxicity study with MITC is available, RMS considers this study as the most appropriate one to be relied upon in risk assessment.
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.2/02.

Data point:	KCA 8.2.2/02
Report author:	[redacted]
Report year:	2019b
Report title:	Sublethal toxic effects on rainbow trout (*Salmo gairdneri* RICH. = *Oncorhynchus mykiss*) of Methylisothiocyanate (MITC) – Statistical Re-analysis
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 210: “Fish Early Life Stage Toxicity Test”, adopted July 26 (Annex 6), 2013
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted.

GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	This study type is no longer required according to the EU data requirements. As this study is available it is presented in this renewal dossier. However, this study is not relied upon in risk assessment.
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report by [redacted], project number [redacted] ([redacted], 1990; KCA 8.2.2/01); for the toxicity of Methylisothiocyanate (MITC) to Rainbow trout (*Oncorhynchus mykiss*), did not provide estimates of the $EC_{10,}$ EC_{20} or EC_{50} for the response variables evaluated as part of the original study. Consequently the data generated in this study were intended to be re-analysed in an attempt to provide these values.

The test design consisted of seven concentrations of the test substance (nominally 0.001, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg test item/L and a control and solvent control). Chemical analysis of the test solutions for the duration of the study showed that the mean measured concentrations ranged from 60 to 107 %, however the chemical was only detectable in the 0.02, 0.05 and 0.3 mg/L treatment groups. It was reported in the analytical report that there were difficulties with contamination and rapid degradation of the analytical water samples. As a result, and in line with the original report, these analyses are based on the nominal concentrations.

Statistical analysis in the original report determined that there were no statistically significant differences in the length and weight data measured at the start at the test before application of the test item to confirm that the fish used in the test were of a similar size. As a result, no additional statistical analysis were performed on these data. There were no significant differences between the control groups for any of the parameters analysed. As a result, all data were analysed in comparison to the pooled control groups.

Statistical analysis of the available data for the survival revealed that the following $LC_{10,}$ LC_{20} and LC_{50} values were reliably calculated:

Table B.2.9.2-111: Mortality of Rainbow trout (*Oncorhynchus mykiss*), exposed to MITC for 28 days under flow-through test conditions, statistical re-analysis based on nominal values

Parameter	28 day Survival using nominal values		
	LC_{10}	LC_{20}	LC_{50}
Value [mg/L]	0.017	0.022	0.035
lower 95 %-cl	0.011	0.015	0.028
upper 95 %-cl	0.022	0.027	0.045

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Statistical recalculation of endpoints for the study KCA 8.2.2/01 ([redacted], 1990)

Original Endpoints:

NOEC (*Oncorhynchus mykiss*, 28 d) = 0.005 mg MITC/L (nominal)

NOEC (*Oncorhynchus mykiss*, 28 d) = 0.004 mg MITC/L (mean measured)

According to PRAPeR 53 (August 2008), for the recalculation of the endpoint in mean measured concentrations, the mean recovery of the concentration 0.05 mg MITC/L of 80 % was used.

The NOEC is based on effects on growth (body weight and body length) and clinical signs.

Endpoints from re-analysis:

EC_{10} (*Oncorhynchus mykiss*, 28 d) = 0.017 mg MITC/L (nominal)

EC_{20} (*Oncorhynchus mykiss*, 28 d) = 0.022 mg MITC/L (nominal)
EC_{50} (*Oncorhynchus mykiss*, 28 d) = 0.035 mg MITC/L (nominal)
Assessment and conclusion by Lainco:
The statistical re-analysis is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
Endpoints:
NOEC (*Oncorhynchus mykiss*, 28 d, flow-through) = 0.005 mg MITC/L (nominal)
NOEC (*Oncorhynchus mykiss*, 28 d, flow-through) = 0.004 mg MITC/L (mean measured)
A statistical re-analysis of the original endpoints was conducted:
EC_{10} (*Oncorhynchus mykiss*, 28 d) = 0.017 mg MITC/L (nominal)
EC_{20} (*Oncorhynchus mykiss*, 28 d) = 0.022 mg MITC/L (nominal)
EC_{50} (*Oncorhynchus mykiss*, 28 d) = 0.035 mg MITC/L (nominal)
Since the OECD Guideline 204 is no longer supported in current aquatic risk assessment, the fish juvenile growth study is considered acceptable as supplementary data and therefore not relied upon.
An alternative fish early life stage toxicity study with MITC is available, RMS considers this study as the most appropriate one to be relied upon in risk assessment.

Data point:	KCA 8.2.2.1/01
Report author:	[redacted]
Report year:	2015
Report title:	Methyl Isothiocyanate (MITC): An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
Report No.:	[redacted]
Document No.:	-
Guidelines followed in study:	OECD 210 (2013): OECD Guideline for Testing of Chemicals No. 210. Fish, Early-life Stage Toxicity Test (2013). US EPA OPPTS Number 850.1400 (1996): US Environmental Protection Agency. Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.1400: Fish Early-Life Stage Toxicity Test (1996). ASTM Standard E 1241-05 (2013): American Society for Testing and Materials. ASTM Standard E 1241-05: Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (2013).
Deviations from current test guideline:	Deviations from current OECD guideline 210 (2013): Dissolved oxygen concentration was ≥ 66 % of air saturation throughout the test, with the exception of Day 27 of the test when the dissolved oxygen in replicate A of the 4.01, 16.3 and 33.5 µg MITC/L treatment groups were 58 %, 58 % and 50 % of air saturation, respectively. The mixing chambers and splits were cleaned and the dissolved oxygen measurement was repeated and confirmed that the dissolved oxygen was ≥ 72 % of air saturation (≥ 5.9 mg/L O_2). Since no additional stress or adverse effects were noted in the fish at the time the dissolved oxygen decline or after the problem was corrected, this slight decline in dissolved oxygen had no impact on the study results or interpretation of the results.
Previous evaluation:	No, not previously submitted at EU level
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities

Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (letter of co-ownership by AMVAC and Taminco is included, study may be used by Taminco without restriction for registration purposes) (no formal letter of access by Lainco is included, the full study was submitted by Lainco)

Study Summary:

The 33 day (5 days hatch and 28 days post-hatch) chronic toxicity of Methyl Isothiocyanate (MITC) to early-life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Eighty fertilized embryos, < 24 hour old were exposed to a control, solvent control (0.1 mL HPLC-grade dimethylformamide/L), and nominal concentrations of the test chemical of 2.19, 4.38, 8.75, 17.5 and 35.0 µg MITC/L.
Samples of the test solutions collected during the test had measured concentrations that ranged from 81.9 % to 104 % of nominal concentrations. The mean measured test concentrations were 2.00, 4.01, 7.74, 16.3 and 33.4 µg MITC/L, which represented 91 %, 92 %, 88 %, 93 % and 95 % of nominal concentrations, respectively. Nevertheless, the toxicological endpoints were evaluated using mean measured concentrations.
There were no statistically significant differences in hatching success between the negative and solvent control groups. A statistically significant decrease in hatching success was observed in the highest treatment group of 33.4 µg MITC/L, compared to the pooled control. A statistically significant decrease in survival was observed in the two highest treatment groups of 16.3 and 33.4 µg MITC/L, compared to the pooled control. Growth data from the 16.3 and 33.4 µg MITC/L treatment groups were excluded from the statistical analysis of growth endpoints due to statistically significant reductions in survival. There was no statistically significant reduction in total length among fish in any of the MITC treatment groups compared to the pooled controls or in wet or dry weight in comparison to the solvent control. Overall, the most sensitive endpoint was survival.
According to the results of this early-life stage fathead minnow test, the overall NOEC value (33 days) for MITC was determined to be 7.74 µg MITC/L (mean measured) and the EC_{10} value for MITC for the most sensitive parameter (survival) was determined to be 9.29 µg MITC/L (mean measured).
This toxicity study is classified as acceptable and satisfies the guideline requirement for an early-life stage toxicity study with fish.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 56198PJV, chemical purity: 97.2 %
Test species:	Fathead minnow (*Pimephales promelas*)
Age, loading:	Age at study initiation: < 24 hours old embryos Biomass loading at the end of the test: 0.045 g of fish/L of test solution that passed through the test chamber during a 24 hour period (based on the mean wet weight of the negative control group)
Feeding:	Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia* sp.) three times per day during the first seven days of post-hatch. Thereafter, they were fed live brine shrimp nauplii three times per day on weekdays and at least two times per day on weekends. Brine shrimp nauplii were obtained by hatching cysts.

	Fish were not fed for approximately 48 hours prior to the termination of the test to allow for clearance of the digestive tracts before weight measurements were made. To ensure that the feeding rate per fish remained constant, rations were adjusted at least weekly to account for losses due to mortality.
Type of test:	Flow-through toxicity test (11 volume additions of test water in each test chamber per day)
Applied concentrations:	Nominal test concentrations: 0 (control), 0 (solvent control: dimethylformamide 0.1 mL/L), 2.19, 4.38, 8.75, 17.5 and 35.0 µg MITC/L
Number of animals per group:	20 embryos per replicate cup, 4 replicates for the control, for the solvent control and per treatment group
Time of exposure:	33 days (5 days hatch and 28 days post-hatch)
Test conditions:	temperature: 24.6 – 26.2 °C (mean: 25.4 °C) dissolved oxygen: 5.4 – 8.2 mg/L O_2 (mean: 6.8 mg/L O_2) (≥ 66 % of air saturation value) pH: 7.6 – 8.7 (mean: 8.0) total hardness: 128 – 144 mg/L $CaCO_3$ (mean: 136 mg/L $CaCO_3$) photoperiod: 16 hours light and 8 hours dark light intensity: fluorescent light bulbs that emit wavelengths similar to natural sunlight
Test procedure:	Fathead minnow embryos were exposed to a series of 5 test concentrations, a negative control and a solvent control under flow-through conditions. 7 L glass aquaria filled with approximately 5 L of test solution (depth of test water in test chamber was approximately 14.1 cm). Embryos were held in incubation cups constructed from glass cylinders approximately 50 mm in diameter with 425 µm nylon screen mesh attached to the bottom with silicone sealant. The cups were suspended in the water column of each test chamber and attached to a rocker arm. The reciprocating motion of the rocker arm (4 rpm) facilitated circulation of test water around the embryos during incubation. At test initiation, embryos < 24 hours old were impartially distributed to incubation cups and exposed to test solution in the test chambers. After a 5-day embryo hatching period, the larvae were released into the test chambers, where exposure continued during a 28-day post-hatch juvenile growth period. Constant flow-through at a rate to provide approximately 11 volume additions of test water in each test chamber per day. Nominal concentrations: 2.19, 4.38, 8.75, 17.5 and 35.0 µg MITC/L, a control (dilution water) and a solvent control (0.1 mL/L HPLC-grade dimethylformamide) following exploratory range-finding toxicity data
Test item analysis:	Water samples were collected from one test chamber of each treatment and control group two days prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on days 0, 7, 13, 20, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers,

	placed in glass vials with septa and processed immediately for analysis. The analytical method consisted of placing the samples in VOA vials with no head space. Samples were submitted for analysis by gas chromatography with flame ionization detection (GC/FID) using an Archon/Tekmar purge and trap system.
Observations:	Time to hatch, hatching success, growth and survival were observed. Embryos were observed twice on the first day of exposure for mortality and fungus on eggs. Until hatching was complete, observations of embryo mortality were performed once daily. When hatching reached > 90 % in the control groups on day 5 of the test, live larvae were released into their respective test chambers and the post-hatch period began. Any unhatched embryos were kept in the egg cups until they hatched and were released into the test chamber, or until death of the embryos occurred. During the 28-day post-hatch exposure period, the larvae were observed daily to evaluate the numbers of mortalities and the numbers of individuals exhibiting clinical signs of toxicity or abnormal behaviour. From these observations, time to hatch, hatching success, and post-hatch growth and survival were evaluated. Hatching success was calculated as the percentage of embryos that hatched successfully. Post-hatch survival was calculated as the number of larvae surviving to test termination divided by the total number of embryos that hatched successfully. Post-hatch growth of the fathead minnows was evaluated at the conclusion of the 28-day post-hatch exposure period. Total length for each surviving fish was measured to the nearest 1 mm using a metric ruler, and wet and dry weights were measured to the nearest 0.1 mg using an analytical balance. Fish were placed in an oven at 60 °C for approximately 48 hours to obtain dry weight data.
Statistical evaluation:	All statistical tests, including the EC_x, NOEC and LOEC values, were performed using SAS or TOXSTAT software. Data on time to hatch was evaluated by visual interpretation of the data. Test endpoints analysed statistically for the juvenile fish were hatching success, larval survival and growth of larvae that survived to test end (total length, wet weight and dry weight). Data from the negative and solvent control groups for each parameter were compared using an appropriate test (e.g. t-test, $\alpha = 0.05$). No statistically significant differences were detected between the control groups ($p > 0.05$) for hatching success, survival and total length, however, statistically significant differences ($p \leq 0.05$) were detected in wet and dry weight. Therefore, the control data for hatching success, survival and total length were pooled for comparison among the treatment groups. For the wet and dry weight data of the treatment groups were compared to the data from the solvent control only. Growth data from the 17.5 and 35.0 µg MITC/L treatment concentrations were excluded from the statistical analysis of growth endpoints due to statistically significant reductions in survival at test termination.

Hatching success was calculated as the percentage of embryos that hatched successfully. Post-hatch survival was calculated from the number of larvae that survived to test termination as a percentage of the number of embryos that hatched successfully. Hatching success and survival data were considered to be discrete-variable data, while growth data were considered continuous-variable data. Discrete-variable data were analysed using Chi-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference ($\alpha = 0.05$) from the pooled control. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test, and for homogeneity of variance using Levene's tests ($\alpha = 0.01$). Since the data passed the assumptions of normality and homogeneity of variances, those treatments that were significantly different from the pooled control or solvent control means were identified using Dunnett's one-tailed test ($\alpha = 0.05$).

Findings:

Analytical results:

Nominal concentrations selected for use in the study were 2.19, 4.38, 8.75, 17.5 and 35.0 µg MITC/L. The test solutions in the mixing chambers and test chambers appeared clear and colorless during the test, with no evidence of precipitation observed in any control or treatment solution.

The measured concentrations of samples collected to verify the diluter system prior to the test ranged from 77.0 to 100 % of nominal concentrations. Samples of the test solutions collected during the test had measured concentrations that ranged from 81.9 to 104 % of nominal concentrations. When the measured concentrations of test solution samples collected on days 0, 7, 13, 20, 28 and 33 of the test were averaged for each treatment group, the mean measured test concentrations were 2.00, 4.01, 7.74, 16.3 and 33.4 µg MITC/L, which represented 91, 92, 88, 93 and 95 % of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Time to hatch and hatching success:

The majority of fathead minnow embryos in the control and treatment replicates hatched on days 4 and 5 of the test. Hatching reached > 90 % in the control groups on day 5 of the test, at which time the larvae were released to their respective test chambers. A few embryos in the 33.4 µg MITC/L treatment group remained in the incubation chambers until they hatched or died by day 10 of the test. Daily observations of the embryos indicated that there were no apparent differences in time to hatch between the control groups and the four lowest treatment groups, but a slight not noticeable delay in hatching was noted in the 33.4 µg MITC/L treatment group.

Hatching success in the negative and solvent control groups was both 100 %. There were no statistically significant differences in hatching success between the negative and solvent control groups ($p > 0.05$). Therefore, the control data were pooled for comparisons with the treatment groups. Hatching success in the pooled control, 2.00, 4.01, 7.74, 16.3 and 33.4 µg MITC/L

	treatment groups was 100 %, 100 %, 100 %, 99 %, 99% and 78 %, respectively. Fisher's Exact test indicated that the decrease in hatching success in the 33.4 µg MITC/L treatment groups was statistically significant in comparison to the pooled controls ($p \leq 0.05$). Consequently, the NOEC for hatching success was 16.3 µg MITC/L and the LOEC was 33.4 µg MITC/L. The EC_{10}, based on hatching success, was 23.3 µg MITC/L, with the 95 % confidence interval of 20.6 to 29.1 µg MITC/L. The EC_{20}, based on hatching success, was 31.4 µg MITC/L, with the 95 % confidence interval of 25.5 to 33.4 µg MITC/L.
Larval survival and clinical observations:	Larval survival in the negative and solvent control groups was 88 and 94 %, respectively. There were no statistically significant differences in larval survival between the negative and solvent control groups ($p > 0.05$). Therefore, the control data were pooled for comparisons with the treatment groups. Larval survival in the pooled control, 2.00, 4.01, 7.74, 16.3 and 33.4 µg MITC/L treatment groups was 91 %, 90 %, 89 %, 90 %, 51 % and 3.2 %, respectively. Fisher's Exact test indicated there were statistically significant decreases in survival in the 16.3 and 33.4 µg MITC/L in comparison to the pooled controls ($p \leq 0.05$). Consequently, the NOEC for larval survival was 7.74 µg MITC/L and the LOEC was 16.3 µg MITC/L. The EC_{10}, based on survival, was 9.29 µg MITC/L, with the 95 % confidence interval of 3.78 to 9.87 µg MITC/L. The EC_{20}, based on survival, was 11.3 µg MITC/L, with the 95 % confidence interval of 9.76 to 12.2 µg MITC/L. In general, the majority of the fish in the control groups and in the 2.00, 4.01 and 7.74 µg MITC/L treatment groups appeared normal throughout the test. During the test, there were observations of organisms that appeared smaller in comparison to the fish in the control replicates, of fish that were weak, lying on the bottom of the test chamber, discolored (pale), observed with an air bubble in the abdomen and had to swim downward to maintain its position in the water's column, or with lost buoyancy and having to swim upward to maintain its position in the water column. Some fish were also observed with internal hemorrhaging or with morphological deformity (i.e. curled/curved/crooked spine or lost part of left operculum). However, these observations were generally infrequent and some observations for the treatment group fish were comparable to observations in the control. In the 16.3 and 33.4 µg MITC/L treatment groups, the frequency of the sublethal effects increased significantly and appeared to be treatment related.
Growth:	There were no statistically significant differences in total length between the negative and solvent control groups ($p > 0.05$). Therefore, the control total length data were pooled for comparisons with the treatment groups. When the wet and dry weight data of the controls were statistically significant different, the treatment wet and dry weight data were compared to the solvent control. Growth data from the 16.3 and 33.4 µg MITC/L treatment groups were excluded from the statistical

analysis of growth endpoints due to statistically significant reductions in survival. There was no statistically significant reduction in the total length among fish in any of the MITC treatment groups in comparison to the pooled controls or in wet or dry weight in comparison to the solvent control (Dunnett's one-tailed test, $p > 0.05$). Consequently, the NOEC for growth was 7.74 µg MITC/L and the LOEC was 16.3 µg MITC/L.
The EC_{20}, based on growth (measured as total length) was 22.9 µg MITC/L, with the 95 % confidence interval of 22.0 to 23.9 µg MITC/L.
The EC_{10}, based on growth (measured as wet weight) was 14.2 µg MITC/L, with 95 % confidence interval of 12.5 to 16.0 µg MITC/L. The EC_{20}, based on growth (measured as wet weight) was 17.2 µg MITC/L, with 95 % confidence interval of 15.6 to 18.9 µg MITC/L.
The EC_{10}, based on growth (measured as dry weight) was 15.5 µg MITC/L, with 95 % confidence interval of 13.7 to 17.5 µg MITC/L.

Table B.2.9.2.3-112: Measured concentrations of Methyl Isothiocyanate (MITC) in test solution samples

Nominal concentration (µg/L)	Measured concentration (µg/L)[1]							% of nominal concen-tration
	Day 0	Day 7	Day 13	Day 20	Day 28	Day 33	Mean	
control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	-	-
solvent control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	-	-
2.19	2.07	1.93	1.85	2.14	2.08	1.90	2.00	91
4.38	3.80	4.23	4.22	4.01	3.92	3.86	4.01	92
8.75	7.69	7.74	7.52	8.36	7.93	7.17	7.74	88
17.5	16.3	17.3	15.2	17.1	16.4	15.4	16.3	93
35.0	32.3	32.7	30.9	36.3	33.3	34.6	33.4	95

[1] The limit of quantification (LOQ) was 1.00 µg a.s./L, calculated as the product of the concentration of the lowest calibration standard (1.00 µg a.s./L) and the dilution factor of the matrix blank samples (1.00)

Table B.2.9.2.3-113: Summary of hatching success, larval survival and growth of fathead minnow (*Pimephales promelas*), exposed to MITC for 33 days under flow-through conditions

Mean measured concentration (µg MITC/L)	Percent hatching success	Percent survival to day 28 post-hatch	Growth parameters at day 28 post-hatch		
			Mean total length ± SD (mm)[1]	Mean wet weight ± SD (mg)[1, 2, 3]	Mean dry weight ± SD (mg)[1, 2, 3]
negative control	100	88	24.2 ± 0.33	119.9 ± 5.63	25.0 ± 1.19
solvent control	100	94	24.1 ± 0.26	106.1 ± 0.91	21.6 ± 0.65
pooled control	100	91	24.1 ± 0.28	-	-
2.00	100	90	24.1 ± 0.23	116.6 ± 4.34	22.8 ± 0.98
4.01	100	89	24.0 ± 0.51	114.9 ± 4.41	21.9 ± 1.35
7.74	99	90	23.8 ± 0.12	112.4 ± 3.80	21.8 ± 0.95
16.3	99	51	22.3 ± 0.59	93.7 ± 6.78	19.3 ± 1.29
33.4	78*	3.2*	13.5 ± 0.71	28.4 ± 6.29	5.0 ± 1.34

** indicates statistically significant decrease in survival when compared to the pooled control ($p \leq 0.05$)*

[1] the growth data for the 16.3 and 33.4 µg MITC/L treatment groups were excluded from the analysis of growth endpoints due to statistically significant reductions in post-hatch survival

[2] since statistically significant differences in wet and dry weight were noted between the negative and solvent control ($p \leq 0.05$), the treatment data for wet and dry weight were compared to the solvent control

[3] there were no statistically significant reductions in wet or dry weight from the solvent control (Dunnett's one-tailed test, $p > 0.05$)

Table B.2.9.2.3-114: Sublethal effects for fathead minnow (*Pimephales promelas*), exposed to MITC for 33 days under flow-through test conditions

Please refer to Volume 3 B.9 (AS).

Assessment and conclusions:

The 33 day chronic toxicity of MITC to early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions in accordance with OECD 210 (2013) and OPPTS 850.1400.

There were no significant effects on hatching success at mean measured concentrations ≤ 16.3 µg MITC/L and on survival at mean measured concentrations ≤ 7.74 µg MITC/L. Fathead minnows exposed to MITC at mean measured concentrations ≤ 7.74 µg MITC/L had no statistically significant reductions in total length in comparison to the pooled controls or in wet weight and dry weight in comparison to the solvent control.

The 33 day EC_{10} and EC_{20} values for the most sensitive parameter (survival) were determined to be 9.29 µg MITC/L (95 % confidence intervals: 3.78 – 9.87 µg MITC/L) and 11.3 µg MITC/L (95 % confidence intervals: 9.76 – 12.2 µg MITC/L), respectively, based on mean measured test concentrations. The overall 33 day NOEC value based on survival, length, fresh weight and dry weight was determined to be 7.74 µg MITC/L (mean measured) and the LOEC value was 16.3 µg MITC/L (mean measured).

This toxicity study is classified as acceptable and satisfies the guideline requirement for an early life stage toxicity study with fish.

Table B.2.9.2.3-115: Toxicity endpoints for fathead minnow (*Pimephales promelas*) early life stages, exposed to MITC after exposure of 33 days under flow-through conditions (based on mean measured concentrations)

Mean measured concentration (µg MITC/L)					
Endpoint	**Hatchability**	**Post-hatch survival**	**Length**	**Fresh weight [a]**	**Dry weight [a]**
NOEC	16.3	7.74	7.74	7.74	7.74
LOEC	33.4	16.3	16.3	16.3	16.3
EC_{10} ± 95% CI	23.3 (20.6-29.1)	9.29 (3.78-9.87)	NA	14.2 (12.5-16.0)	15.5 (13.7-17.5)
EC_{20} ± 95% CI	31.4 (25.5-33.4)	11.3 (9.76-12.2)	22.9 (22.0-23.9)	17.2 (15.6-18.9)	NA

NA = not applicable; since the calculated ECx value was extrapolated beyond the data range used in the calculation and/or the 95 % confidence interval contains zero or was overly wide.
[a] Since the wet and dry weight data of the negative control and solvent control were different, only solvent control data were included in the determination of ECx.
CI: Confidence interval

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
NOEC (*Pimephales promelas*, 33 d, growth, post-hatch survival) = 7.74 µg MITC/L (mean measured)
EC_{10} (*Pimephales promelas*, 33 d, survival) = 9.24 µg MITC/L (mean measured)
EC_{20} (*Pimephales promelas*, 33 d, survival) = 11.3 µg MITC/L (mean measured)
Analytical method:
This study is performed in compliance with the guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore considered acceptable.
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
NOEC (*Pimephales promelas*, 33 d, flow-through, growth, post-hatch survival) = 7.74 µg MITC/L (mean measured)
EC_{10} (*Pimephales promelas*, 33 d, flow-through, survival) = 9.24 µg MITC/L (mean measured)
EC_{20} (*Pimephales promelas*, 33 d, flow-through, survival) = 11.3 µg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is compared with the current guidance.
The validity criteria of OECD Guideline 210 were met:
- the dissolved oxygen concentration should be > 60 % of the air saturation value throughout the test (measured: ≥ 66 % of air saturation value)
- the water temperature should not differ by more than ± 1.5 °C and should be within the temperature ranges specified for the test species (25 ± 1.5 °C for *Pimephales promelas*) (measured: 24.6 – 26.2 °C (mean: 25.4 °C)
- the analytical measure of the test concentrations is compulsory (see Table above)
- overall survival of fertilised eggs and post-hatch success in the controls and solvent controls should be greater than or equal to the limits defined for the test species (minimum hatching success of 70 % and minimum post-hatch success of 75 % for *Pimephales promelas*) (measured: 100 % hatching success and 88 – 94 % post-hatch success)

Deviations from current OECD guideline 210 (2013):

Dissolved oxygen concentration was ≥ 66 % of air saturation throughout the test, with the exception of Day 27 of the test when the dissolved oxygen in replicate A of the 4.01, 16.3 and 33.5 µg MITC/L treatment groups were 58 %, 58 % and 50 % of air saturation, respectively. The mixing chambers and splits were cleaned and the dissolved oxygen measurement was repeated and confirmed that the dissolved oxygen was ≥ 72 % of air saturation (≥ 5.9 mg/L O2). Since no additional stress or adverse effects were noted in the fish at the time the dissolved oxygen decline or after the problem was corrected, this slight decline in dissolved oxygen had no impact on the study results or interpretation of the results.

Therefore, this study is considered acceptable.

The analytical method used could be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/84 (Taminco) and KCA 4.1.2/16 (Lainco), for further details).

Endpoints:

NOEC (*Pimephales promelas*, 33 d, flow-through, growth, post-hatch survival) = 7.74 µg MITC/L (mean measured)

EC_{10} (*Pimephales promelas*, 33 d, flow-through, survival) = 9.24 µg MITC/L (mean measured)

EC_{20} (*Pimephales promelas*, 33 d, flow-through, survival) = 11.3 µg MITC/L (mean measured)

This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for fish.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. 2 chronic toxicity studies with aquatic invertebrates are available for MITC.

Studies with MITC

Data point:	KCA 8.2.5.1/01
Report author:	[redacted]
Report year:	2001
Report title:	Methylisothiocyanate – Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS.
Report No.:	99/0547/51/2
Document No.:	2002/1000250
Guidelines followed in study:	OECD guideline 202, Part II (1984) EEC Guideline XI/691/86, Draft 4 DIN 38 412 (draft, 1981) US EPA 660/3-75-009
Deviations from current test guideline:	Deviations from current OECD guideline 211 (2012): Light intensity: 2 – 7 µE/(m² s) (recommendation: 15 – 20 µE/(m² s) M4 medium: 0.020 mg/L $SeSO_4$ (recommendation: 0.002 mg/L) Analytical measurements: In the highest concentration and in medium test concentrations (recommendation: highest and lowest test concentrations) Control coefficient of variation for the mean number of living young per surviving animal was 32.2 % (recommendation: ≤ 25 %) Deviations have no impact on the results of the study. The testing with the control substance potassium dichromate and the reproduction rate of breeding of the daphnia shows no deviation from the existing limits.
Previous evaluation:	Yes, evaluated and accepted in the original DAR (August 2007, updated August 2010)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (data out of protection) (original Sponsor: BASF)

Study Summary:

The lethal and sublethal effects of Methylisothiocyanate (MITC) on *Daphnia magna* were evaluated in a 21 day toxicity test performed under semi-static conditions. The investigation was carried out following OECD guideline 202 (1984).
10 replicates with one *Daphnia magna* per concentration were exposed to nominal concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg MITC/L. Daphnids were observed regularly to determine the number of produced young, dead organisms and other signs of toxicity in each treatment group.

Mean measured concentrations of 6.25, 25 and 100 µg/L were between 87 % and 88 %. Therefore, effect concentrations were based on nominal concentrations. All validity criteria according to the current OECD 211 were fulfilled. In the control group, no mortality was observed and first young were observed at day 8. At the two highest test concentrations of 50 and 100 µg MITC/L all daphnids died. Significantly reduced numbers of living offspring per parent were observed in the 25 µg MITC/L treatment group. In the original study summary, the NOEC for reproduction was 12.5 µg MITC/L. The corresponding LOEC was 25 g/L. Following review in the DAR (2007), the NOEC for reproduction was considered to be 6.25 µg MITC/L due to non-statistically significant but biologically relevant effects at 12.5 µg MITC/L. The corresponding LOEC was 12.5 µg/L.
The 48 hour EC_{50} was 134 µg MITC/L (based on nominal concentrations). The 48 hour NOEC was 39 µg MITC/L.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: 340401/1, chemical purity: not reported
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	First instar, 2 - 24 hours old (3rd breed of parent animal)
Feeding:	Green algae (*Desmodesmus subspicatus*) The algae were separated from culture medium by centrifugation, resuspended in daphnid's medium (M4) and daphnids were fed this concentrate (maximum: 0.3 mL/50 mL/day)
Type of test:	Semi-static toxicity test (medium renewal each Monday, Wednesday and Friday)
Applied concentrations:	Nominal test concentrations: 0 (control), 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg MITC/L
Dilution medium:	Elendt M4 medium
Number of organisms per group:	1 daphnid per replicate, 10 replicates for the control and per treatment group
Time of exposure:	21 days In-life dates: August 1st to 22nd 2001
Test conditions:	temperature: 20.3 – 20.6 °C dissolved oxygen: 4.5 – 9.5 mg/L O_2 (49 – 103 % of air saturation value) pH: 7.0 – 8.2 total hardness: 2.20 – 3.20 mmol/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 2 – 7 µE/(m^2 s)
Test procedure:	The lethal and sublethal effects of Methylisothiocyanate (MITC) on *Daphnia magna* were evaluated in a 21 day toxicity test performed under semi-static conditions. MITC test concentrations were selected based on the results of an acute test with a derived EC_{50} (48 h) of 0.032 mg/L. The study was performed in ten glass beakers per test concentration, each containing 50 mL test solution. 10 replicates with 1 *Daphnia magna* per concentration were exposed to nominal concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg MITC/L. In addition, 10 x 1 *Daphnia magna* were exposed to test medium without test substance (control).

Daphnids were daily fed live green algae (*Desmodesmus subspicatus*).
For test solution preparation, the test substance was pulverized in a mortar. The stock solution (100 mg/L) was stirred in M4 medium for about 20 minutes at 20 ± 2°C, put for approximately 2 minutes in an ultra-sonic bath and then again stirred in M4 medium for about 20 minutes. Test concentrations were established by diluting the stock solution with M4 medium. Medium renewal and removal of young from test beakers was performed three times per week. Additionally, the control substance potassium dichromate was tested under non-GLP conditions.

Test item analysis: The analyses were carried out as a separate study. The analytical verification of the substance was performed with nominal MITC concentrations of 6.25, 25 and 100 µg/L via capillary gas chromatography, evaluation by the internal standard method. Samples for analysis were taken in the 1st, 2nd and 3rd week of the test. For each concentration, the freshly prepared test solution (unstocked) and the corresponding 48 hour or 72 hour old test solution (unstocked) were analysed.
The samples were analysed by gas chromatography with flame ionization detection (GC-FPD).

Observations: Daphnids were observed daily to determine the number of dead organisms and other signs of toxicity in each treatment group. Three times per week Daphnids were checked for embryos and aborted eggs. Water temperature was measured continuously during the whole test period in a separate vessel close to the test vessels. Dissolved oxygen and pH were measured in the new test solutions at the start of the test until day 14 at each change of the test solutions and in 48 hour or 72 hour old solution in one parallel at each concentration.

Statistical evaluation: For the statistical evaluation of the LOEC and NOEC Duncan´s multiple range test was used.

Findings:

Analytical results: At test initiation and in old medium, the recoveries for MITC were in a range between 80 and 96 %. Mean measured concentrations of 6.25, 25 and 100 µg MITC/L were between 87 and 88 %. Therefore, effect concentrations were based on nominal concentrations.
Detailed results of measured MITC concentrations are presented in the table below.

Adult mortality and reproduction: In the control group, first young were observed at day 8. In the highest test concentration, in which the daphnids produced young (25 µg MITC/L), the first young were also observed at day 8. No mortality was observed in the control groups. At the two highest test concentrations of 50 and 100 µg MITC/L all daphnids died. Significant reduced numbers of living offspring per parent were observed in the 25 µg MITC/L treatment group.

The effects of MITC on *Daphnia magna* mortality and reproduction are shown in the table below.

Table B.2.9.2.3-116: Mean measured concentrations of MITC of two samples taken each from freshly prepared treatment solutions (new) and before treatment solution renewal (old) in the 1st, 2nd and 3rd week of the *Daphnia magna* reproduction test

Nominal concentration of test item (µg MITC/L)	Measured concentrations (µg MITC/L)						Mean	Percent of nominal (%)
	Week 1		Week 2		Week 3			
	New	Old	New	Old	New	Old		
control	< 2	< 2	< 2	< 2	< 2	< 2	-	-
6.25	6	5.5	5	5	5	6	5.42	87
25	23	22	21.5	21.5	22.5	22.5	22.2	87
100	90	87.5	85	84	90	88.5	87.5	88

Table B.2.9.2.3-117: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions

Evaluation criteria	Control	Nominal test concentration (µg MITC/L)							
		0.78	1.56	3.13	6.25	12.5	25	50	100
Mortality of the parent daphnids (%)	0	0	20	0	0	10	30	100	100
Mean number of living young per surviving parent animal after 21 day exposure period (mean ± SD)	74.9 ± 23.4	82.2 ± 33.6	71.1 ± 19.4	65.2 ± 24.4	81.5 ± 30.6	52.7 ± 31.8	45.4 ± 25.1*	-	-
Mean number of dead young per surviving parent animal after 21 day exposure period	10.1	9.0	8.8	12.4	8.8	10.1	13.6	-	-
Mean number of aborted subitane eggs per surviving parent animal after 21 day exposure period	1.0	0.9	1.1	0.6	0.8	0.6	1.6	-	-

** statistically significantly different from the control*

Assessment and conclusions:

In the original study report, the 21 day NOEC for reproduction of *Daphnia magna* exposed to MITC was 12.5 µg/L based on nominal concentration. The corresponding LOEC was 25 µg/L.

The following RMS comment was included in the DAR (2007):
The notifier study report proposed a NOEC of 0.0125 mg MITC/L based on statistics. However, a high variation in the results was observed for the control and all treatment groups (high standard deviation and high coefficient of variation). The number of living young per surviving parent animal after the 21 day exposure period ranged from 47 to 106 in the control. The sum of all replicates of living young per surviving parent animal after 21 day exposure period was 749 in the control, 815 at the treatment level of 6.25 µg MITC/L and only 474 at the treatment level of 12.5 µg MITC/L. Although the chronic effects at 12.5 µg MITC/L were not statistically significantly different from the control, the RMS considered them biologically relevant. By precautionary principle, the NOEC was set at 6.25 µg MITC/L.

Therefore, the 21 day NOEC for reproduction of *Daphnia magna* exposed to MITC was considered to be 6.25 µg/L based on nominal concentration. The corresponding LOEC was 12.5 µg/L.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable. Following review in the DAR (2007), the NOEC for reproduction was lowered from 12.5 µg MITC/L to 6.25 µg MITC/L due to non-statistically significant but biologically relevant effects at 12.5 µg MITC/L.
Endpoints:
NOEC (*Daphnia magna*, 21 d) = 6.25 µg MITC/L (nominal)
Analytical method:
Method validation not fit for purpose.
Assessment and conclusion by Lainco:
The study is mentioned in the dossier of the applicant Lainco.
EU-agreed endpoint (EFSA Journal 2011; 9(9): 2334) :
NOEC (*Daphnia magna*, 21 d, semi-static) = 0.00625 mg MITC/L (nominal)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 211 were met:
- the mortality of the parent animals (female *Daphnia*) does not exceed 20 % at the end of the test (measured: 0 %)
- the mean number of living offspring produced per parent animal surviving at the end of the test is ≥ 60 (measured: 74.9)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 211 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could not be sufficiently validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and therefore it is uncertain to consider it "fit for purpose" based on the available data (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/78, for further details).
Originally the applicant proposed the NOEC of 0.0125 mg MITC/L. Although the chronic effects at 0.0125 mg MITC/L were not statistically significantly different from the control, the RMS considered them biologically relevant. By precautionary principle, the NOEC was set at 0.00625 mg MITC/L.
NOEC (*Daphnia magna*, 21 d, semi-static) = 0.00625 mg MITC/L (based on nominal concentrations)
Note: A statistical re-analysis of the endpoints is presented under KCA 8.2.5.1/02.
Due to the uncertainties mentioned, RMS considers the endpoint not the most appropriate for risk assessment. During renewal, a new chronic study was submitted (KCA 8.2.5.1/03) with better study design (flow-through) and thus reliable endpoint for the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.5.1/02
Report author:	[redacted]
Report year:	2019c
Report title:	Methylisothiocyanate – Determination of the Chronic Effect on the Reproduction of the Water Flea *Daphnia magna* STRAUS – Statistical Re-analysis.

Report No.:	CEA.2037
Document No.:	-
Guidelines followed in study:	Not conducted according to any specific regulatory guideline, but the following were consulted: OECD Guidelines for Testing of Chemicals, No. 211: "*Daphnia magna* Reproduction Test", adopted, 2012. OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006. OECD Guidelines for Testing of Chemicals, No. 210: "Fish Early Life Stage Toxicity Test", adopted July 26 (Annex 6), 2013
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted.
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (GLP not relevant to statistical re-analysis)
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (no data protection claimed)

Study Summary:

The report from BASF Aktiengesellschaft Project Number: 99/0547/51/2 ([redacted] 2001; KCA 8.2.5.1/01) for the chronic toxicity of Methylisothiocyanate to *Daphnia magna* did not provide estimates of the EC_{10}, EC_{20} or EC_{50} for the response variables evaluated as part of the original study. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.
The test used nominal concentrations of Methylisothiocyanate at 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/L. Chemical analysis of the test solutions showed that the mean measured concentrations ranged from 80 % to 96 % of the nominal values. As a result, and in line with the original report, these analyses are based on the nominal values.
The following parameters were analysed statistically:

- Cumulative number of offspring per parent after 21 days
- Cumulative number of offspring per surviving parent after 21 days
- Cumulative number of dead offspring after 21 days
- Cumulative number of aborted eggs after 21 days
- Cumulative mortality of parent *Daphnia magna* after 21 days

Statistical analyses of the available data for **Number of Cumulative Offspring per Parent after 21 Days** revealed that the following EC_{10}, EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.3-118: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions, statistical re-analysis for cumulative number of offspring per parent after 21 days, based on nominal values

Parameter	EC_{10}	EC_{20}	EC_{50}
Value [µg/L]	7.218	9.970	18.496
lower 95 %-cl	n.d.	n.d.	n.d.
upper 95 %-cl	n.d.	n.d.	n.d.

Statistical analyses of the available data for **Number of Cumulative Offspring per Surviving Parent after 21 Days** revealed that the following EC_{10}, EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.3-119: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions, statistical re-analysis for cumulative number of offspring per surviving parent after 21 days, based on nominal values

Parameter	EC_{10}	EC_{20}	EC_{50}
Value [µg/L]	n.d.	n.d.	70.256
lower 95 %-cl	n.d.	n.d.	n.d.
upper 95 %-cl	n.d.	n.d.	n.d.

Statistical analyses of the available data for **Number of Cumulative Dead Offspring after 21 days** revealed that the following $EC_{10,}$ EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.3-120: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions, statistical re-analysis for cumulative number of dead offspring after 21 days, based on nominal values

Toxicity Metric	EC_{10}	EC_{20}	EC_{50}
Value [µg/L]	1.123	26.502	n.d.
lower 95 %-cl	n.d.	8.351	n.d.
upper 95 %-cl	3.094	n.d.	n.d.

Statistical analyses of the available data for **Cumulative Number of Aborted Eggs after 21 Days** revealed that the following $EC_{10,}$ EC_{20} and EC_{50} values were reliably calculated:

Table B.2.9.2.3-121: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions, statistical re-analysis for cumulative number of aborted eggs after 21 days, based on nominal values

Toxicity Metric	EC_{10}	EC_{20}	EC_{50}
Value [µg/L]	n.d.	n.d.	n.d.
lower 95 %-cl	n.d.	n.d.	n.d.
upper 95 %-cl	n.d.	n.d.	n.d.

Statistical analyses of the available data for **Mortality of Parent *Daphnia magna* after 21 Days** revealed that the following $LC_{10,}$ LC_{20} and LC_{50} values were reliably calculated:

Table B.2.9.2.3-122: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under semi-static test conditions, statistical re-analysis for cumulative mortality of parent *Daphnia magna* after 21 days, based on nominal values

Toxicity Metric	LC_{10}	LC_{20}	LC_{50}
Value [µg/L]	4.966	8.292	22.116
lower 95 %-cl	n.d.	n.d.	n.d.
upper 95 %-cl	n.d.	n.d.	n.d.

Assessment and conclusion by the applicant:

Assessment and conclusion by Eastman (Taminco):

The study is acceptable.

Statistical recalculation of endpoints for the study KCA 8.2.5.1/01 ([redacted], 2001)

Following review in the DAR (2007), for the original study analysis the NOEC for reproduction was lowered from 12.5 µg MITC/L to 6.25 µg MITC/L due to non-statistically significant but biologically relevant effects at 12.5 µg MITC/L.

Original Endpoints:
NOEC (*Daphnia magna*, 21 d) = 6.25 µg MITC/L (nominal)
Endpoints from re-analysis:
Cumulative number of offspring per parent after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = 7.218 µg MITC/L (nominal)
EC_{20} (*Daphnia magna*, 21 d) = 9.970 µg MITC/L (nominal)
EC_{50} (*Daphnia magna*, 21 d) = 18.496 µg MITC/L (nominal)
Cumulative number of offspring per surviving parent after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = n.d.
EC_{20} (*Daphnia magna*, 21 d) = n.d.
EC_{50} (*Daphnia magna*, 21 d) = 70.256 µg MITC/L (nominal)
Cumulative number of dead offspring after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = 1.123 µg MITC/L (nominal)
EC_{20} (*Daphnia magna*, 21 d) = 26.502 µg MITC/L (nominal)
EC_{50} (*Daphnia magna*, 21 d) = n.d.
Cumulative number of aborted eggs after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = n.d.
EC_{20} (*Daphnia magna*, 21 d) = n.d.
EC_{50} (*Daphnia magna*, 21 d) = n.d.
Cumulative mortality of parent *Daphnia magna* after 21 days:
LC_{10} (*Daphnia magna*, 21 d) = 4.966 µg MITC/L (nominal)
LC_{20} (*Daphnia magna*, 21 d) = 8.292 µg MITC/L (nominal)
LC_{50} (*Daphnia magna*, 21 d) = 22.116 µg MITC/L (nominal)
Assessment and conclusion by Lainco:
The statistical re-analysis is not mentioned in the dossier of the applicant Lainco.

Assessment and conclusion by the RMS:
Endpoints:
NOEC (*Daphnia magna*, 21 d, semi-static) = 0.00625 mg MITC/L (nominal)
A statistical re-analysis of the original endpoints was conducted:
Cumulative number of offspring per parent after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = 7.218 µg MITC/L (nominal)
EC_{20} (*Daphnia magna*, 21 d) = 9.970 µg MITC/L (nominal)
EC_{50} (*Daphnia magna*, 21 d) = 18.496 µg MITC/L (nominal)
Cumulative number of offspring per surviving parent after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = n.d.
EC_{20} (*Daphnia magna*, 21 d) = n.d.
EC_{50} (*Daphnia magna*, 21 d) = 70.256 µg MITC/L (nominal)
Cumulative number of dead offspring after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = 1.123 µg MITC/L (nominal)
EC_{20} (*Daphnia magna*, 21 d) = 26.502 µg MITC/L (nominal)
EC_{50} (*Daphnia magna*, 21 d) = n.d.
Cumulative number of aborted eggs after 21 days:
EC_{10} (*Daphnia magna*, 21 d) = n.d.
EC_{20} (*Daphnia magna*, 21 d) = n.d.
EC_{50} (*Daphnia magna*, 21 d) = n.d.
Cumulative mortality of parent *Daphnia magna* after 21 days:
LC_{10} (*Daphnia magna*, 21 d) = 4.966 µg MITC/L (nominal)

LC_{20} (*Daphnia magna*, 21 d) = 8.292 µg MITC/L (nominal) LC_{50} (*Daphnia magna*, 21 d) = 22.116 µg MITC/L (nominal) Due to the uncertainties mentioned, RMS considers the endpoint not the most appropriate for risk assessment. During renewal, a new chronic study was submitted (KCA 8.2.5.1/03) with better study design (flow-through) and thus reliable endpoint for the risk assessment for aquatic invertebrates.

Data point:	KCA 8.2.5.1/03
Report author:	[redacted]
Report year:	2019c
Report title:	Methyl isothiocyanate (MITC) – Effect on Survival, Reproduction and Growth of *Daphnia magna* in a Flow-Through Test over Three Weeks.
Report No.:	20180076
Document No.:	-
Guidelines followed in study:	OECD No. 211 (2012) Method C.20 of Commission Regulation (EC) No. 440/2008
Deviations from current test guideline:	Deviations from current OECD guideline 211 (2012): None
Previous evaluation:	No, not previously submitted.
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Study is acceptable
Study owner:	Taminco BV, US TF (original Sponsor: Kanesho Soil Treatment, letter of access by Taminco is included, study may be used by Taminco in Europe only) (original Sponsor: Kanesho Soil Treatment, letter of access by Lainco is included, study may be used by Lainco in Europe only)

Study Summary:

The impact of the test item Methyl isothiocyanate (MITC) on the survival, growth (body length) and reproduction rate of *Daphnia magna* was investigated in a flow-through test over 21 days following the OECD Guidelines for Testing of Chemicals, No. 211 (2012): "*Daphnia magna* Reproduction Test" and the Commission Regulation (EC) No 440/2008, C.20: "*Daphnia magna* Reproduction Test" (amended by Commission Regulation (EC) No. 2017/735).
In this flow-through test, 10 daphnids each were exposed to five different test item concentrations over a period of 21 days. Mortality and reproduction of the daphnids were recorded. At test end the body length of the parental daphnids was measured.
The nominal test concentrations tested were 6.25, 12.5, 25, 50 and 100 µg MITC/L. Additionally a control and a solvent control group were tested in parallel. The test concentrations selected were based on a flow-through range-finding test.
The concentrations of Methyl isothiocyanate (MITC) were analytically determined in all test media and the solvent control.
In the samples from the analysed test media the mean measured concentrations of Methyl isothiocyanate (MITC) over the 21-days test period were in the range of 81 – 85 % of the nominal values (8 measurements). The analytical results showed that under the flow-through conditions applied the

concentrations of the test item Methyl isothiocyanate (MITC) could be maintained sufficiently constant during the test period of 21 days. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test concentration. The biological results are based on nominal and on mean measured test item concentrations.
In conclusion, taking into account the effects of Methyl isothiocyanate (MITC) on survival, growth and reproduction of the test animals, the overall 21 day NOEC was 25 µg/L nominal (21.1 µg/L mean measured), since no toxic effects were observed at the daphnids at that test concentration.
The 21 day LOEC was 50 µg/L nominal (40.4 µg/L mean measured), due to the statistically significantly reduced reproduction rate, the inhibitory effect on the growth of the parental daphnids and the observed mortality at this test concentration.
The EC_{10} for the reproduction rate was 43 µg MITC/L based on nominal concentrations and 35 µg MITC/L based on mean measured concentrations, respectively. The EC_{20} for the reproduction rate was 50 µg MITC/L based on nominal concentrations and 42 µg MITC/L based on mean measured concentrations, respectively.
The EC_{10} for body length was 81 µg MITC/L based on nominal concentrations and 65 µg MITC/L based on mean measured concentrations, respectively. The EC_{20} for body length was 111 µg MITC/L based on nominal concentrations and 90 µg MITC/L based on mean measured concentrations, respectively.

Materials and methods:

Test substance:	Methyl isothiocyanate (MITC), batch no: STBB1308V, chemical purity: 99.6 % (according to certificate of analysis)
Test species:	Waterflea (*Daphnia magna*)
Age of organisms:	First instar, < 24 hours old
Feeding:	Green algae (*Desmodesmus subspicatus*) The concentration of algae in the test media flowing through the test vessels was 0.3 mg C/L at the start of the test and was adapted successively to the need of the growing daphnids with 0.5 mg C/L at days 1 and 2, 0.6 mg C/L during days 3 to 7 and 0.7 mg C/L durin days 8 to 21.
Type of test:	Flow-through toxicity test (19-fold theoretical test medium exchange rate per day)
Applied concentrations:	Nominal test concentrations: 0 (control), solvent control (50 µL/L N,N-dimethylformamide), 6.25, 12.5, 25, 50 and 100 µg MITC/L
Dilution medium:	Elendt M7 medium
Number of organisms per group:	1 daphnid per replicate, 10 replicates for the control, for the solvent control and per treatment group
Time of exposure:	21 days
Test conditions:	temperature: 19.1 – 20.3 °C dissolved oxygen: 7.8 – 8.3 mg/L O_2 pH: 7.8 – 7.9 total hardness: 250 mg/L $CaCO_3$ photoperiod: 16 hours light and 8 hours dark light intensity: 10 – 12 µE/(m^2 s)
Test procedure:	The test was run under flow-through conditions using a computer-controlled dosing system. The study was started with 10 daphnids per treatment. Each test animal was kept individually in a flow-

	through test beaker. The test animals were randomly distributed to the test vessels. The test duration was 21 days. Based on the results of a range-finding test, the following nominal concentrations were tested in the main test: 6.25, 12.5, 25, 50 and 100 µg MITC/L. Additionally a control (test water without test item) and a solvent control (test water containing the solvent) were tested in parallel.
Test item analysis:	For the analysis of the test item concentrations in the test media, duplicate samples from the test media of all test concentrations and from the solvent control were taken at the start of the test (day 0) and at seven further sampling dates distributed over the test period. The duplicate analytical samples were taken from the different replicates of the respective test concentration. Immediately after sampling, to each test medium sample about 20 g of sodium chloride were added. Then, the samples were extracted with internal standard solution. The organic phase was separated and stored frozen until analysis was performed. The concentrations of the test item in the test media were analytically determined in one of the duplicate samples from the solvent control and all test concentrations from all sampling dates. The analytical method used is gas chromatography with mass spectrometric detection (GC/MS).
Observations:	The test replicates were observed daily for immobility of adults or other visible abnormalities. On the same dates, the test replicates were observed for the number of living and dead offspring and for the presence of aborted eggs. Offspring was separated from the adult at the day of observation by transferring the adult daphnia in a new test vessel by means of a wide bore glass pipette. The offspring was counted and was disposed thereafter. The body length of surviving adults was determined at the end of the test by measuring the daphnids from the top of the head to the basis of the spina with the use of a binocular microscope. The adults were disposed after evaluation. The reproduction rate for the controls and the test concentrations of nominal 6.25 to 25 µg MITC/L was calculated as the total number of living offspring produced per parent female surviving until the end of the test (i.e. 10 surviving daphnids). At the test concentrations 50 and 100 µg MITC/L only 9 and 2 daphnids, respectively, survived until the end of the test. As this parental mortality was supposed to be related to a toxic effect of the test item (i.e. part of the concentration effect curve), the dead parental daphnids have not been excluded from the calculation of the mean reproduction rate, but the total number of offspring produced at these test concentrations was divided by 10, i.e. the number of introduced daphnia (following the recommendations for calculation of the reproduction rate of the test guideline). Water temperature, oxygen concentration and pH were measured alternately in one the replicates of all test concentrations with

	surviving daphnids and in the controls at the start of the test and at least two times per week thereafter. Additionally, the water temperature in the control was monitored continuously by a data logger. The appearance of the application solutions and the test media in the mixing vessels and test vessels was checked each working day.
Statistical evaluation:	Statistical analysis was performed using ToxRat Professional®, Version 3.3.0. The results obtained from the solvent control were tested for statistically significant differences compared to the control by Student-t Tests. The test item treatments were compared for statistically significant differences to the solvent control. The following statistical tests were applied: Mortality/immobility: The NOEC was determined directly from the raw data without statistical evaluation. Reproduction rate: Williams t-test, one-sided smaller, $\alpha = 0.05$ Body length: Williams t-test, one-sided smaller, $\alpha = 0.05$ The 21 day EC_{10} values and EC_{20} values for the inhibition of the reproduction rate and body length were calculated by the 3-parametric, non-linear, normal CDF (Cumulative Distribution Function).

Findings:

Analytical results:	In the samples from the analysed test media the mean measured concentrations of Methyl isothiocyanate (MITC) over the 21 days test period were in the range of 81 – 85 % of the nominal values during the test period (8 measurements). The nominal concentrations of 6.25, 12.5, 25, 50 and 100 µg MITC/L correspond to mean measured concentrations of 5.3, 10.5, 21.1, 40.4 and 80.5 µg MITC/L, respectively. The analytical results showed that under the flow-through conditions applied the concentrations of the test item Methyl isothiocyanate (MITC) could be maintained sufficiently constant during the test period of 21 days. The mean measured test item concentrations were calculated as arithmetic means over all measurements per test concentration. The application solutions, the test media in the mixing chambers of the dosing units and the test media in the test vessels appeared to be clear solutions throughout the test period. No remarkable observations (e.g. precipitation or turbidity) were made.
Mortality of parental daphnids:	In the control, the solvent control and all test concentrations up to and including 25 µg MITC/L nominal (21.1 µg MITC/L mean measured), the survival of the test animals at the end of the test was 100 %. At the test concentration of 50 µg MITC/L nominal (40.4 µg MITC/L mean measured) one daphnid was dead at day 12 of the test. At the highest test concentration, only 20 % of the daphnids survived. The LOEC for survival was determined to be the test concentration of 50 µg MITC/L nominal (40 µg MITC/L mean

	measured) with 10 % mortality. This low mortality was considered to be due to a toxic effect of the test item (i.e. the beginning of the concentration effect curve) as at the next higher test concentration of 100 µg MITC/L nominal (80.5 µg MITC/L mean measured) the mortality rate was 80 %. Furthermore, the toxic effect of the test item at 50 µg MITC/L nominal (40.4 µg MITC/L mean measured) was also demonstrated by the reduced reproduction rate and body length. The NOEC for mortality was determined to be 25 µg MITC/L nominal (21.1 µg MITC/L mean measured).
Reproduction rate:	The first young offspring released from their parent animals were recorded in the control, the solvent control and at all test concentrations up to and including 50 µg MITC/L nominal (40.4 µg MITC/L mean measured) at observation day 8. Thus, the time of the first brood was not affected by the test item up to and including the highest test concentration of 50 µg/L nominal (40.4 µg/L mean measured). At 100 µg MITC/L nominal (80.5 µg MITC/L mean measured), first offspring was observed at day 11. The mean reproduction rate of the daphnids in the control was 139 ± 8 living offspring per surviving adult (mean ± standard deviation) and was 143 ± 13 living offspring in the solvent control. No inhibitory effect of the test item on the mean reproduction rate was determined up to and including the test concentration of 25 µg MITC/L nominal (21.1 µg MITC/L mean measured). At the test concentration of 50 µg MITC/L nominal (40.4 µg MITC/L mean measured), the offspring was statistically significantly reduced to 84 % of the solvent control. At the highest concentration of 100 µg MITC/L nominal (80.5 µg MITC/L mean measured) the mean reproduction rate was 17 % of the solvent control. The NOEC for mean reproduction rate was determined to be the test concentration of 25 µg MITC/L (21.1 µg MITC/L mean measured). The LOEC was determined to be 50 µg MITC/L nominal (40.4 µg MITC/L mean measured).
Body length:	The mean body length of the daphnids in the control was 4.8 ± 0.08 mm and was 4.8 ± 0.06 in the solvent control. No inhibitory effect of the test item on the body length was determined up to and including the test concentration of 25 µg MITC/L nominal (21.1 µg MITC/L mean measured). At the test concentration of 50 µg MITC/L nominal (40.4 µg MITC/L mean measured), the body length was statistically significantly reduced to 97 % of the solvent control. At the highest concentration of 100 µg MITC/L nominal (80.5 µg MITC/L mean measured) the mean body length was 84 % of the solvent control value. The NOEC for mean body length was determined to be the test concentration of 25 µg MITC/L nominal (21.1 µg MITC/L

	mean measured). The LOEC was determined to be 50 µg MITC/L nominal (40.4 µg MITC/L mean measured).
Visible abnormalities:	With the exception of the reported toxic effects, no visible abnormalities were observed at the daphnids during the test.

Table B.2.9.2.3-123: Summary of effects on *Daphnia magna*, exposed to MITC for 21 days under flow-through test conditions

Evaluation criteria	Control	solvent control	Nominal test concentration (µg MITC/L)				
			6.25	12.5	25	50	100
			Mean measured test concentration (µg MITC/L)				
			5.3	10.5	21.1	40.4	80.5
Surviving test animals on day 21 (%)	100	100	100	100	100	90	20
Mean number of living offspring produced per adult after 21 days of exposure (mean ± SD)	139.4 ± 8	142.7 ± 13	152 ± 7	143 ± 25	164 ± 6	119 ± 31*	25 ± 24*
Body length (mm) of the surviving adults after 21 days of exposure (mean ± SD)	4.77 ± 0.084	4.76 ± 0.064	4.76 ± 0.064	4.76 ± 0.064	4.77 ± 0.056	4.64 ± 0.13*	4.00 ± 0*

** statistically significantly different compared to the solvent control (Williams t-test, one sided smaller, α = 0.05)*

Assessment and conclusions:

Taking into account the effects of Methyl isothiocyanate (MITC) on survival, growth and reproduction of the test animals, the overall 21 day NOEC was 25 µg MITC/L nominal (21.1 µg MITC/L mean measured), since no toxic effects were observed at the daphnids at that test concentration. The 21 day LOEC was 50 µg MITC/L nominal (40.4 µg MITC/L mean measured), due to the statistically significantly reduced reproduction rate, the inhibitory effect on the growth of the parental daphnids and the observed mortality at this concentration.
The EC_{10} for reproduction rate was 43 µg MITC/L (95 % confidence interval: 36 – 50 µg MITC/L) based on nominal concentrations and 35 µg MITC/L (95 % confidence interval: 31 – 41 µg MITC/L) based on mean measured concentrations, respectively. The EC_{20} for reproduction rate was 50 µg MITC/L (95 % confidence interval: 42 – 59 µg MITC/L) based on nominal concentrations and 42 µg MITC/L (95 % confidence interval: 37 – 46 µg MITC/L) based on mean measured concentrations, respectively.
The EC_{10} for body length was 81 µg MITC/L (95 % confidence interval: 75 – 87 µg MITC/L) based on nominal concentrations and 65 µg MITC/L (95 % confidence interval: 61 – 70 µg MITC/L) based on mean measured concentrations, respectively. The EC_{20} for body length was 111 µg MITC/L (95 % confidence interval: 101 – 122 µg MITC/L) based on nominal concentrations and was 90 µg MITC/L (95 % confidence interval: 81 – 98 µg MITC/L) based on mean measured concentrations, respectively.

Assessment and conclusion by the applicant:
Assessment and conclusion by Eastman (Taminco):
The study is acceptable.
Endpoints:
NOEC (*Daphnia magna*, 21 d) = 21.1 µg MITC/L (mean measured)
LOEC (*Daphnia magna*, 21 d) = 40.4 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, reproduction rate) = 35 µg MITC/L (mean measured)

EC_{20} (*Daphnia magna*, 21 d, reproduction rate) = 42 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, body length) = 65 µg MITC/L (mean measured)
EC_{20} (*Daphnia magna*, 21 d, body length) = 90 µg MITC/L (mean measured)
Assessment and conclusion by Lainco:
The study is acceptable.
Endpoints:
NOEC (*Daphnia magna*, 21 d) = 21.1 µg MITC/L (mean measured)
LOEC (*Daphnia magna*, 21 d) = 40.4 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, reproduction rate) = 35 µg MITC/L (mean measured)
EC_{20} (*Daphnia magna*, 21 d, reproduction rate) = 42 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, body length) = 65 µg MITC/L (mean measured)
EC_{20} (*Daphnia magna*, 21 d, body length) = 90 µg MITC/L (mean measured)

Assessment and conclusion by the RMS:
The study is considered to be in line with the current guidance.
The validity criteria of OECD Guideline 211 were met:

- the mortality of the parent animals (female *Daphnia*) does not exceed 20 % at the end of the test (measured: 0 % in the control and 0 % in the solvent control)
- the mean number of living offspring produced per parent animal surviving at the end of the test is ≥ 60 (measured: 139.4 in the control and 142.7 in the solvent control)

Also, analytical measurement of test concentrations was performed, as recommended by OECD Guideline 211 when reporting the data.
Therefore, this study is considered acceptable.
The analytical method used could be validated according to the EU Guidance SANCO/3029/99 rev. 4 on analytical validation, and is therefore considered "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – KCA 4.1.2/87 (Taminco) and KCA 4.1.2/18 (Lainco), for further details).
NOEC (*Daphnia magna*, 21 d) = 21.1 µg MITC/L (mean measured)
LOEC (*Daphnia magna*, 21 d) = 40.4 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, reproduction rate) = 35 µg MITC/L (mean measured)
EC_{20} (*Daphnia magna*, 21 d, reproduction rate) = 42 µg MITC/L (mean measured)
EC_{10} (*Daphnia magna*, 21 d, body length) = 65 µg MITC/L (mean measured)
EC_{20} (*Daphnia magna*, 21 d, body length) = 90 µg MITC/L (mean measured)
This study is considered acceptable and since the test item is MITC, which is driving the aquatic risk assessment, the endpoint is relied upon in the risk assessment for aquatic invertebrates.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Please refer to 2.9.2.2.2 above.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No studies submitted.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 2.9.4.2.1-1 Summary of information on acute aquatic toxicity relevant for classification of metam

Method	Species	Test material	Results[1]	Remarks	Reference
Acute fish study based on US EPA 72-1 Not GLP	*Lepomis macrochirus*	Metam-sodium, Purity: 42.2%, Batch no.: ZH 130 585	LC_{50} > 0.522 mg a.s./L (mean measured)	96 h static 10 fish/treatment 1 replicate/ treatment	CA8.2.1/03 [redacted], 1986 CA8.2.1/04 [redacted], 2019 a
Acute daphnia study based on EC 440/2008 Part C Method2, OECD 202 GLP	*Daphnia magna*	Metam-sodium, Purity: 51.99 %, Batch no.: E4227	**EC_{50} = 0.166 mg a.s./L** (mean measured)	48 h semi-static 5 daphnids/ replicate, 4 replicates/ Treatment	CA8.2.4.1/03 [redacted] 2013
Algal growth inhibition study based on EC 92/69/EEC Part C Method 3, OECD 201 GLP	*Pseudokirchne-riella subcapitata*	Metam-sodium, Purity: 44.7 %, Batch no.: B3698	E_yC_{50} = 0.118 mg a.s./L E_yC_{10} = 0.061 mg a.s./L E_bC_{50} = 0.117 mg a.s./L E_bC_{10} = 0.0482 mg a.s./L E_rC_{50} = 0.339 mg a.s./L E_rC_{10} = 0.0779 mg a.s./L $NOEC_y$ = $NOEC_b$ = 0.0378 mg a.s./L $NOEC_r$ = 0.0813 mg a.s./L (mean measured)	72 h static Initial cell count: 1 x 10^4 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/03 [redacted], 2011

Based on the available acute (short-term) aquatic toxicity studies, aquatic invertebrates were identified as the most sensitive species with an EC_{50} of 0.166 mg a.s./L.

Based on this endpoint, the following classification for metam according to CLP is proposed:

- **Aquatic acute category 1 (based on EC_{50} aquatic invertebrates ≤ 1 mg/L)**
- **M-factor = 1 (based on 0.1 mg/L < EC_{50} < 1 mg/L)**
- **H400**

Table 2.9.4.2.1-2 Summary of information on acute aquatic toxicity relevant for classification of MITC

Method	Species	Test material	Results[1]	Remarks	Reference
Acute fish study based on US EPA 72-1 and OECD 203 GLP	*Oncorrhynchus mykiss*	MITC, Purity: 99.6%, Batch no.: 408208/1	LC_{50} = 0.0531 mg MITC/L (mean measured)	96 h semi-static 7 fish/replicate 1 replicate/ treatment	CA8.2.1/10 [redacted], 2002
Acute invertebrate study based on OECD 202, US EPA OPPTS 850.1010 GLP	*Hyalella azteca*	MITC, Purity: 97.2 %, Batch no.: 56198PJV	**LC_{50} = 0.0038** mg MITC/L (mean measured)	48 h static 5 amphipods/ replicate 4 replicates/ treatment	CA8.2.4.2/06 [redacted], 2014a
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} = 0.189 mg MITC/L E_rC_{10} = 0.076 mg MITC/L E_yC_{50} = 0.091 mg MITC/L E_yC_{10} = 0.051 mg MITC/L E_bC_{50} = 0.0933 mg MITC/L E_bC_{10} = 0.0456 mg MITC/L (mean measured)	72 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/09 [redacted], 2018b CA8.2.6.1/10 [redacted], 2020d
Lemna growth inhibition study based on OECD 221, EC 2016/266 Method C26	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (frond numbers) = 0.43 mg MITC/L E_rC_{10} (frond numbers) = 0.18 mg MITC/L E_rC_{50} (dry weight) = 0.29 mg MITC/L E_rC_{10} (dry weight) = 0.13 mg MITC/L (mean measured)	7 d flow-through Inoculation with 3 randomly selected colonies per vessel (12 fronds/ 3 colonies) 3 replicates/ control 3 replicates/ solvent control 3 replicates/ treatment	CA8.2.7/03 [redacted], 2019c

Based on the available acute (short-term) aquatic toxicity studies, aquatic invertebrates were identified as the most sensitive species with an EC_{50} of 0.0038 mg MITC/L.

Based on this endpoint, the following classification for MITC according to CLP is proposed:

- **Aquatic acute category 1 (based on EC_{50} aquatic invertebrates ≤ 1 mg/L)**
- **M-factor = 100 (based on 0.001 mg/L < EC_{50} < 0.01 mg/L)**
- **H400**

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 2.9.2.4.2-1 Summary of information on long-term aquatic toxicity relevant for classification of metam

Method	Species	Test material	Results[1]	Remarks	Reference
Algal growth inhibition study based on EC 92/69/EEC Part C Method 3, OECD 201 GLP	*Pseudokirchne-riella subcapitata*	Metam-sodium, Purity: 44.7 %, Batch no.: B3698	E_yC_{50} = 0.118 mg a.s./L E_bC_{50} = 0.117 mg a.s./L E_rC_{50} = 0.339 mg a.s./L $NOEC_y$ = $NOEC_b$ = 0.0378 mg a.s./L $NOEC_r$ = 0.0813 mg a.s./L (mean measured)	72 h static Initial cell count: 1 x 10^4 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/03 [redacted], 2011

No chronic studies with fish or aquatic invertebrates and metam are available. Therefore, the surrogate approach is used to determine the chronic classification for these organism groups. The lowest LC_{50} for fish is > 0.522 mg a.s./L and the lowest EC50 for aquatic invertebrates is 0.166 mg a.s./L (see Table 2.9.4.2.1-1). Since both these values are below 1 mg/L, and metam is not rapidly degradable, this surrogate approach indicates that metam should be classified as Aquatic chronic category 1.

For algae, a study is available, with a NOEC value of 0.0378 mg a.s./L. As this value is below 0.1 mg/L, this also indicates that metam should be classified as Aquatic chronic category 1.

Based on the above, the following classification for metam according to CLP is proposed:
- **Aquatic chronic category 1 (based on NOEC algae ≤ 0.1 mg/L)**
- **M-factor = 1 (based on 0.01 mg/L < NOEC ≤ 0.1 mg/L** and **Not Rapidly Degradable**
- **H410**

Table 2.9.2.4.2-2 Summary of information on long-term aquatic toxicity relevant for classification of MITC

Method	Species	Test material	Results[1]	Remarks	Reference
Chronic fish early life stage toxicity study based on OECD 210 GLP	*Pimephales promelas*	MITC, Purity: 97.2 %, Batch no.: 56198PJV	NOEC = 0.00774 mg MITC/L EC_{10} = 0.00924 mg MITC/L (mean measured)	33 d flow-through 20 embryos/ replicate 4 replicates/ control 4 replicates/ treatment	CA8.2.2.1/01 [redacted] 2015
Chronic daphnia reproductive toxicity study based on OECD 211, EC 440/2008 Method C20	*Daphnia magna*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	NOEC = 0.0211 mg MITC/L EC_{10} = 0.035 mg MITC/L (mean measured)	21 d flow-through 1 daphnid/ replicate 10 replicates/ treatment	CA8.2.5.1/03 [redacted] 2019c

Method	Species	Test material	Results[1]	Remarks	Reference
Algal growth inhibition study based on OECD 201, EU 2016/266 Method C3 GLP	*Pseudokirchne-riella subcapitata*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} = 0.189 mg MITC/L E_rC_{10} = 0.076 mg MITC/L E_yC_{50} = 0.091 mg MITC/L E_yC_{10} = 0.051 mg MITC/L E_bC_{50} = 0.0933 mg MITC/L E_bC_{10} = 0.0456 mg MITC/L (mean measured)	72 h static Initial cell count: 5000 cells/mL 6 replicates/ control 3 replicates/ treatment	CA8.2.6.1/09 [redacted]., 2018b CA8.2.6.1/10 [redacted], 2020d
Lemna growth inhibition study based on OECD 221, EC 2016/266 Method C26	*Lemna gibba*	MITC, Purity: 99.6 %, Batch no.: STBB1308V	E_rC_{50} (frond numbers) = 0.43 mg MITC/L E_rC_{10} (frond numbers) = 0.18 mg MITC/L E_rC_{50} (dry weight) = 0.29 mg MITC/L E_rC_{10} (dry weight) = 0.13 mg MITC/L (mean measured)	7 d flow-through Inoculation with 3 randomly selected colonies per vessel (12 fronds/ 3 colonies) 3 replicates/ control 3 replicates/ solvent control 3 replicates/ treatment	CA8.2.7/03 [redacted] 2019c

Based on the available long-term aquatic toxicity studies, fish were identified as the most sensitive species with a lowest NOEC of 0.00774 mg MITC/L.

Based on this information, the following classification for MITC according to CLP is proposed:

- **Aquatic chronic category 1 (based on NOEC fish ≤ 0.1 mg/L)**
- **M-factor = 10 (based on 0.001 mg/L < NOEC ≤ 0.01 mg/** and **Not Rapidly Degradable**
- **H410**

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Metam:

Classification:

Aquatic Acute category 1	(based on EC_{50} aquatic invertebrates ≤ 1 mg/L)
H400	
M-factor = 1	(based on 0.1 mg/L < $L(E)C_{50}$ ≤ 1 mg/L)
Aquatic Chronic category 1	(based on NOEC algae ≤ 0.1 mg/L)
H410	
M- factor = 1	(based on 0.01 mg/L < NOEC ≤ 0.1 mg/L)

Labelling:

GHS pictogram:	yes
Signal word:	warning
Hazard assessment:	H410 Very toxic to aquatic life with long lasting effects
Precautionary statements:	Prevention – P273 Avoid release to the environment Response – P391 Collect spillage Disposal – P501 Dispose of contents / container to … in accordance with local regulations

MITC;

Classification:

Aquatic Acute category 1	(based on EC_{50} aquatic invertebrates ≤ 1 mg/L)
H400	
M-factor = 100	(based on 0.001 mg/L < $L(E)C_{50}$ ≤ 0.01 mg/L)
Aquatic Chronic category 1	(based on NOEC fish ≤ 0.1 mg/L)
H410	
M- factor = 10	(based on 0.001 mg/L < NOEC ≤ 0.01 mg/L)

Labelling:

GHS pictogram:	yes
Signal word:	warning
Hazard assessment:	H410 Very toxic to aquatic life with long lasting effects
Precautionary statements:	Prevention – P273 Avoid release to the environment Response – P391 Collect spillage Disposal – P501 Dispose of contents / container to … in accordance with local regulations

2.9.3 Summary of effects on arthropods

2.9.3.1 Bees

Studies on the acute oral and contact toxicity and the chronic toxicity to adult honey bees, and the chronic toxicity to honeybee larvae of metam and the representative formulations (Metam Sodium 51% SL and Metam Na 510 SL) have not been submitted. However, such studies are not considered required, as direct acute and chronic exposure to honey bee adults and larvae following the proposed application of metam to bare soil is not expected.

Two studies on the acute toxicity of MITC through vapour exposure are available. The endpoints of all available studies are summarised in Table 2.9.3.1-1.

Table 2.9.3.1-1: Summary of bee toxicity data for MITC

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Honeybees					
Honeybee (*Apis mellifera*)	MITC	48h acute toxicity test (vapour exposure for 4h)	LC_{50} (inhalation, 4h)	24 ppm (equivalent to 70770 µg MITC/m³)	CA8.3.1.1/01 [redacted], 2016
Honeybee (*Apis mellifera*)	MITC	48h acute toxicity test (vapour exposure for 48h)	LC_{50} (inhalation, 48h)	**> 4710 µg MITC/m³**	CA8.3.1.1/02 [redacted], 2019

bold **-** *values used in the risk assessment*

2.9.3.2 Other non-target arthropods

Standard laboratory studies with the indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* have not been submitted for the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL or the active metabolite MITC. As a general soil fumigant, metam (and MITC) is known to have insecticidal properties and adverse effects on the two indicator non-target arthropod species would be expected based on a worst-case HQ scenario with data from standard laboratory tests. In addition, the traditional test design for this type of Tier 1 studies, in which arthropods are exposed to dried residues on glass plates, is not suitable for volatile compounds such as metam/MITC applied as soil fumigants.

Instead, an extended laboratory study and higher tier field studies were submitted to address the risk to non-target arthropods. These studies focussed on soil-dwelling arthropods, as these will be the main group of non-target arthropods that is exposed following application of Metam Sodium 51% SL and Metam Na 510 SL.

For the active substance impurity DMTU, Tier 1 laboratory studies with the two standard indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* are available.

An overview of the available toxicity data is shown in Table 2.9.3.2-1

Table 2.9.3.2-1: Summary of arthropod toxicity data on metam and DMTU in laboratory and field studies

Species	Test substance	Study type	Endpoint	Reference
Tier 1 laboratory studies				
Aphidius rhopalosiphi	DMTU	48h, laboratory test, artificial substrate, 2D exposure to adults	Mortality: LR_{50} = 103.4 g DMTU/ha NOER = 37.5 g DMTU/ha Reproduction:	CA8.3.2.1/01 [redacted], 2018

Species	Test substance	Study type	Endpoint	Reference
			No effects on reproduction > 21% up to 75 g DMTU/ha (NOER = 75 g DMTU/ha)	
Typhlodromus pyri	DMTU	7 days, laboratory test, artificial substrate, 2D exposure to nymphs	Mortality: LR_{50} = 17.9 g DMTU/ha NOER = 13.3 g DMTU/ha Reproduction: No effects on reproduction > 30% up to 13.3 g DMTU/ha (NOER = 13.3 g DMTU/ha)	CA8.3.2.2/01 [redacted] 2018
Extended laboratory studies				
Aleochara bilineata	Metam sodium 510 SL	28 days , extended laboratory study, natural soil containing aged residues (55 days)	14.6% effect on reproduction of *Aleochara bilineata* following 28 day laboratory exposure of adults to aged field soil (field soil exposed to 608.4 kg metam sodium/ha, aged for 55 days under full field conditions)	CP10.3.2.2/01 [redacted], 2002
Field studies				
Natural non-target arthropod fauna	The results of this study indicate that metam-sodium, applied through soil injection followed by sealing of the soil with a roller, at a rate of 612 kg a.s./ha has initial adverse effects on arthropods living in the soil and on the soil surface. The most important taxa showed recovery in the field within one year: - For the soil samples 0% taxa showed a recovery > 1 year. The arthropod taxa that would be expected to be most at risk from soil injected treatment such as metam-sodium would be the small and relatively immobile soil-dwelling taxa such as Collembola, soil mites, larvae of soil-dwelling beetles (e.g. Aleocharinae and some Carabidae). Most of these groups showed recovery by the end of autumn in the year of application of metam sodium. Numbers of the mite taxa Gamasida and Oribatida had recovered to a level no longer statistically significantly different from the control by the end of the season (November 2008). However, in spring 2009, the densities in the metam-sodium treated plots were again lower than the control on one sampling moment (10 April). In the additional argumentation by [redacted] (2010; CP103.2.4/02) it is argued that for Gamasida, this difference was only observed for the adults, and that this did not result in considerable reductions of juvenile populations. Therefore, it is unlikely that Gamasida populations would be reduced later in the season. Moreover, on the last sampling moment in May 2009, differences compared to the control were not statistically significant and had reduced to levels below 50%, which is considered ecologically acceptable. This implies Gamasida recovery by the end of the study. For Oribatida, no additional argumentation was provided. However, the situation is similar to that of Gamasida. In the original study report, the authors also conclude recovery for this taxon by the end of the study, as on the last sampling moment in May 2009 differences compared to the control were not statistically significant and had			CP10.3.2.4/01 [redacted] 2010

Species	Test substance	Study type	Endpoint	Reference
	reduced to levels below 50%. - For the pitfall samples only 2% taxa showed a recovery > 1 year. Of all the sampled taxa the only taxon that did not show full recovery within one year was Heteroptera. Heteroptera contributed largely to the delayed response pattern in the community response. Heteroptera are primarily foliage-dwelling arthropods and therefore not strongly associated with bare soil, which is the GAP for the application of metam. Any effects of metam-sodium on Heteroptera did not begin to occur until 4 months after treatment. Such a long delay to onset of effects is due to an indirect effect, either the decline in numbers of a suitable prey (for example aphids) on the carrot plants or decline in quality of a host plant (for phytophagous Heteroptera). The numbers of Heteroptera in metam-sodium treated plots were not statistically different from controls in samples taken the year after treatment. Despite being reported as not having shown recovery, the data shows that no difference was observed in absolute numbers just before and one year after treatment. Overall, for the most important soil inhabiting and soil surface dwelling species, the results indicate that recovery occurred within one year after application.			

2.9.4 Summary of effects on non-target soil meso- and macrofauna

2.9.4.1 Earthworms

Standard laboratory studies with the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL, or the active metabolite MITC with earthworms have not been submitted. As a general soil fumigant, adverse effects of metam (and MITC) on earthworms would be expected based on a worst-case TER scenario with data from standard laboratory tests. Instead, higher tier field studies were submitted to address the risk to earthworms.

In the literature review conducted in accordance with article 8(5) of Regulation (EC) No. 1107/2009 (see Volume 3 (CA), Section B.9.11), an acute toxicity study on earthworms was found.

For the active substance impurity DMTU, a Tier 1 chronic laboratory toxicity study with earthworms is available.

An overview of all available toxicity data is shown in Table 2.9.4.1-1

Table 2.9.4.1-1: Summary of earthworm toxicity data on metam and DMTU in laboratory and field studies

Species	Test substance	Study type	Endpoint	Toxicity value	Reference
Standard laboratory studies					
Eisenia fetida	Metam sodium	14 days, acute test, 10% organic mater	Artificial soil: LC_{50} Natural soil: LC_{50}	0.72 mg a.s./kg soil dw 0.67 mg a.s./kg soil dw	CA8.4.1/01 [redacted]., 2017
Eisenia fetida	DMTU	56 days, reproduction test, 10% organic mater	EC_{50} EC_{20} EC_{10} NOEC	344 mg DMTU/kg soil dw 100 mg DMTU/kg soil dw 52 mg DMTU/kg soil dw **95 mg DMTU/kg soil dw**	CA8.4.1/02 [redacted], 2018
Field studies					
Natural earthworm populations	This field study was performed in Germany. Metam-sodium 510 g/L was applied through soil injection at 152.1 kg a.s./ha and 608.4 kg a.s./ha (actual applied rates). Immediately after treatment, the soil was treated with a rotavator to incorporate the test item up to a depth of 25 cm, and compressed with a roller. Two weeks after application, the soil was re-opened with a cultivator. Five weeks after treatment, *Phacelia tanacetifolia* was sown on the plots. Based on the results of this study, there are still significant effects on the earthworm abundance at both the treatment of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha) **compared to the untreated control** 12 months after application. However, there are also clear differences between the untreated control and the agricultural control (same general cultivation methods as in the metam-sodium treatments) in terms of earthworm abundance, which indicates that the mechanical soil cultivation has an influence on the ability of an earthworm population to recover. Therefore, the effects of metam-sodium and its metabolite MITC should rather be assessed in comparison to the agricultural control. This comparison will filter out any effects on earthworms due to soil cultivation, rather than by exposure to the test item. The results of this study indicate that one year after treatment of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), the earthworm abundance was 103.5 % and 85.2 % of the abundance in the **agricultural control**, respectively. The earthworm biomass was 123.7 % and 85.8 % of the biomass in the agricultural control one year after application of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), respectively. Amongst the collected earthworms, the most abundant species was *Aporrectodea caliginosa*. $4^{1/2}$ months after treatment with 300 and 1200 L Metam-Sodium/ha				CP 10.4.1.2/01 [redacted], 2002

Species	Test substance	Study type	Endpoint	Toxicity value	Reference
	(equivalent to 152.1 and 608.4 kg a.s./ha), the abundance of *Aporrectodea caliginosa* was comparable to that in the agricultural control. The abundance of juvenile earthworms one year after application of 300 L Metam-Sodium/ha (equivalent to 152.1 kg a.s./ha) was comparable to the agricultural control, whereas at 1200 L Metam-Sodium/ha (equivalent to 608.4 kg a.s./ha) it was still statistically significantly lower. In conclusion, based on the results from this study, the earthworm population in the field can be considered recovered one year after application of 152.1 kg a.s./ha. Although there are indications of an ongoing recovery one year after application of 608.4 kg a.s./ha, full recovery was not observed for this treatment.				
Natural earthworm populations	This field study was performed in Northern France. Raisan 51 (Metam-sodium 510 g/L) was applied through soil injection at 196.7 kg a.s./ha and 286.11 kg a.s./ha (actual applied rates). Immediately after treatment, the soil was ploughed to a depth of 25 cm, and compressed with a roller. During the remainder of the study, no additional ploughing was performed. Three weeks after treatment, the test area was sown with rye-grass. The results of this study indicate that, **compared to the untreated control**, the abundance and biomass of the earthworms was statistically significant reduced at each sampling point up to 12 months after application for the 286.11 kg a.s./ha treatment. Only at the last sampling point (18 months after application), the difference was no longer significant, indicating full recovery. For the 196.70 kg a.s./ha treatment, there was a significant reduction of earthworm abundance at each sampling point after application, except at 18 months after application. For the earthworm biomass, there was a significant difference at 1 and 4 months after application, but no longer at 12 and 18 months after application. In addition to an untreated control, a soil incorporated water control was also included, in which the same soil cultivation was applied as in the test item treatments (i.e. soil injection of water followed immediately by ploughing to a dept of 25 cm and compression with a roller). As there are clear differences between the untreated control and the soil incorporated water control in terms of earthworm abundance, there are indications that the mechanical soil cultivation has an influence on the ability of an earthworm population to recover. Therefore, the effects of metam-sodium and its metabolite MITC should rather be assessed in comparison to the soil incorporated water control. This comparison will filter out any effects on earthworms due to soil cultivation, rather than by exposure to the test item. When **compared to the soil incorporated water control**, there were no significant differences in earthworm abundance and biomass for any of the sampling occasions. However, for the sampling one month after application, the abundance and biomass were both 0% of the values found in the soil incorporated water control for the 286.11 kg metam sodium/ha treatment. For the 196.70 kg metam sodium/ha treatment, the abundance and biomass were only 6.7 and 4.6% of the values of the soil incorporated water control, respectively. The fact that this difference was not statistically significant, is likely due to the overall low number of earthworms observed at this sampling occasion. Nevertheless, these results indicate that there is a biologically relevant effect on earthworm abundance and biomass directly after application, which would be expected based on the properties of metam sodium. From the sampling 4 months after application, earthworm abundance and biomass in both the 286.11 and 196.70 kg metam sodium/ha treatments were again at the same level as in the soil incorporated water control, indicating full recovery.				CP 10.4.1.2/03 (Lainco) [redacted], 2012
Natural earthworm populations	This field study was performed in East-Central France. Metam-sodium 510 g/L was applied through soil injection at 292.1 kg a.s./ha, 407.5 kg a.s./ha and 562.7 kg a.s./ha (actual applied rates). Immediately after treatment, the soil was ploughed to a depth of 25 cm, and compressed with a roller. 5 weeks after treatment, the test area was mulched, superficially ploughed and sown with rye-grass.				CP 10.4.1.2/03 (Taminco) [redacted], 2015

Species	Test substance	Study type	Endpoint	Toxicity value	Reference
	The results of this study indicate that, **compared to the untreated control**, the abundance and biomass of the earthworms (all species) was statistically significantly reduced at each sampling point up to 12 months after application for the three treatment levels (292.1, 407.5 and 562.7 kg metam sodium/ha). At the sampling 16 months after application, the earthworm abundance was still statistically significantly reduced for the 407.5 and 562.7 kg metam sodium/ha treatments, but not longer for the 292.1 kg metam sodium/ha treatment. At the same sampling, the biomass was only statistically significantly reduced at the highest tested treatment rate of 562.7 kg metam sodium/ha. At the samplings 21 and 24 months after application, both the abundance and biomass were not statistically significantly different from the untreated control for any of the three treatments. This indicates that recovery occurred within 16 months after application of 292.1 kg a.s./ha, and within 21 months after application of 407.5 and 562.7 kg a.s./ha. In addition to an untreated control, a **soil injected water control** was also included, in which the same soil cultivation was applied as in the test item treatments (i.e. soil injection of water followed immediately by ploughing to a dept of 25 cm and compression with a roller). However, in contrast to the other available field studies, no difference was observed between the untreated control and the soil injected water control for any of the samplings after application. However, in the pre-application sampling, the mean abundance in the soil injected water control was significantly higher compared to the untreated control and the metam sodium treatments. Therefore, it is possible that the higher pre-treatment abundance in the soil injected water control masked the effect of the soil cultivation.				

2.9.4.2 Other non-target soil macro-organisms

Standard laboratory studies with the indicator species *Hypoaspis aculeifer* and *Folsomia candida* have not been submitted for the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL, or the active metabolite MITC. As a general soil fumigant, adverse effects of metam and/or MITC on the two soil indicator species would be expected based on a worst-case TER scenario with data from standard laboratory tests. Instead, higher tier field studies were submitted to address the risk to non-target soil meso- and macrofauna. As these studies addressed both effects on non-target arthropods living on the soil surface and macro-organisms living in soil, these are the same as those mentioned under Section 2.9.3.2.

For the active substance impurity DMTU, Tier 1 laboratory studies with the two standard indicator species Hypoaspis aculeifer *and* Folsomia candida *are available.*

Table B.2.9.4-1: Summary of non-target soil meso- and macrofauna (other than earthworms) toxicity data on metam and DMTU in laboratory and field studies

Species	Test substance	Study type	Endpoint	Toxicity value	Reference
Standard laboratory studies					
Folsomia candida	DMTU	28 days, reproduction test, 5% organic mater	EC_{50} EC_{20} EC_{10} NOEC	> 40 mg DMTU/kg soil dw > 40 mg DMTU/kg soil dw > 10 mg DMTU/kg soil dw **40 mg DMTU/kg soil dw**	CA8.4.2/01 [redacted], 2018a
Hypoaspis aculeifer	DMTU	14 days, reproduction test, 5% organic mater	EC_{50} EC_{20} EC_{10} NOEC	321 mg DMTU/kg soil dw 229 mg DMTU/kg soil dw 192 mg DMTU/kg soil dw **100 mg DMTU/kg soil dw**	CA8.4.2/02 [redacted]., 2018b

Species	Test substance	Study type	Endpoint	Toxicity value	Reference
Field studies					
Natural non-target arthropod fauna	The results of this study indicate that metam-sodium, applied through soil injection followed by sealing of the soil with a roller, at a rate of 612 kg a.s./ha has initial adverse effects on arthropods living in the soil and on the soil surface. The most important taxa showed recovery in the field within one year: - For the soil samples 0% taxa showed a recovery > 1 year. The arthropod taxa that would be expected to be most at risk from soil injected treatment such as metam-sodium would be the small and relatively immobile soil-dwelling taxa such as Collembola, soil mites, larvae of soil-dwelling beetles (e.g. Aleocharinae and some Carabidae). Most of these groups showed recovery by the end of autumn in the year of application of metam sodium. Numbers of the mite taxa Gamasida and Oribatida had recovered to a level no longer statistically significantly different from the control by the end of the season (November 2008). However, in spring 2009, the densities in the metam-sodium treated plots were again lower than the control on one sampling moment (10 April). In the additional argumentation by [redacted] (2010; CP103.2.4/02) it is argued that for Gamasida, this difference was only observed for the adults, and that this did not result in considerable reductions of juvenile populations. Therefore, it is unlikely that Gamasida populations would be reduced later in the season. Moreover, on the last sampling moment in May 2009, differences compared to the control were not statistically significant and had reduced to levels below 50%, which is considered ecologically acceptable. This implies Gamasida recovery by the end of the study. For Oribatida, no additional argumentation was provided. However, the situation is similar to that of Gamasida. In the original study report, the authors also conclude recovery for this taxon by the end of the study, as on the last sampling moment in May 2009 differences compared to the control were not statistically significant and had reduced to levels below 50%. - For the pitfall samples only 2% taxa showed a recovery > 1 year. Of all the sampled taxa the only taxon that did not show full recovery within one year was Heteroptera. Heteroptera contributed largely to the delayed response pattern in the community response. Heteroptera are primarily foliage-dwelling arthropods and therefore not strongly associated with bare soil, which is the GAP for the application of metam. Any effects of metam-sodium on Heteroptera did not begin to occur until 4 months after treatment. Such a long delay to onset of effects is due to an indirect effect, either the decline in numbers of a suitable prey (for example aphids) on the carrot plants or decline in quality of a host plant (for phytophagous Heteroptera). The numbers of Heteroptera in metam-sodium treated plots were not statistically different from controls in samples taken the year after treatment. Despite being reported as not having shown recovery, the data shows that no difference was observed in absolute numbers just before and one year after treatment. Overall, for the most important soil inhabiting and soil surface dwelling species, the results indicate that recovery occurred within one year after application.					CP10.3.2.4/01 [redacted], 2010

2.9.5 Summary of effects on soil nitrogen transformation

Laboratory toxicity studies on the effect of the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL, or the active metabolite MITC on soil nitrogen transformation have not been submitted. As metam is a general soil fumigant, a significant impact on soil microbial activity is inherent to the active substance. Therefore, the notifier proposed not to submit a standard laboratory test according to OECD 216. Instead, a higher tier laboratory study, using aged soil samples collected from a treated field has been submitted to address the risk to soil nitrogen transformation.

In the literature review conducted in accordance with article 8(5) of Regulation (EC) No. 1107/2009 (see Volume 3 (CA), Section B.9.11), several studies on effects of metam sodium on the structure and function of the soil microbial community were found. Although most of these studies were acceptable, and provided useful information, they are only considered as supportive information. The most important reason for this is that in all cases detailed information on the test item (e.g. active substance content in the formulation, type of formulation, batch number, etc.) was lacking. In most cases, the results from these studies could not directly be used in the risk assessment. In general, these published literature studies demonstrated that, following application of metam sodium, there will be an immediate statistically significant effect on the soil microbial populations. However, recovery to pre-treatment levels was found to occur in the median or long term (i.e. after a few weeks or months). In that respect, the results of the published literature studies are generally in line with the outcome of the available guideline studies.

For the active substance impurity DMTU, a Tier 1 laboratory study on the effect on soil nitrogen transformation is available.

An overview of all available toxicity data is shown in Table 2.9.5-1.

Table 2.9.5-1: Summary of data on the effect of metam and DMTU on soil nitrogen transformation and soil respiration

Test system	Test substance	Test soil	Duration of exposure	NOEC	References
N transformation	Metam sodium 507 g/L	Silty sand to loamy sand	184 days	At application rates of 152.1 and 608.4 kg a.s./ha, significant effects on nitrogen and carbon transformation were observed in soil collected from the field 1 to 56 day after application. In soil collected from the field site 102 and 184 days after application, the observed effects were no longer statistically significant. The effects of metam-sodium and its metabolite MITC on nitrogen and carbon transformation were therefore transient and the processes were not drastically impaired.	CA8.5/01 [redacted]., 2002
C transformation					
N transformation	DMTU	Loamy sand	43 days	16.5 mg DMTU/kg soil dw	CA8.5/11 [redacted], 2018

2.9.6 Summary of effects on terrestrial non-target higher plants

Standard laboratory studies with the active substance metam or the representative formulations Metam Sodium 51% SL and Metam Na 510 SL with non-target plants have not been submitted. Instead, studies with the active metabolite MITC have been provided, as this is the main molecule that non-target plants will be exposed to. Studies on the effects of MITC on the seedling emergence of different plant species have been submitted, as well as a study on the vegetative vigour of different plant species following vapour exposure to MITC.

An overview of the available toxicity data is shown in table 2.9.6-1

Table 2.9.6-1: Summary of plant toxicity data on MITC and DMTU in laboratory studies

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value [a]	Reference
10 non-target plant species	MITC	Vegetative vigour test	EC_{50} NOEC	**0.5 ppm MITC (equivalent to 1502 µg MITC/m³)** < 0.53 ppm MITC (equivalent to < 1592 µg MITC/m³)	CA8.6.2/01 [redacted], 2013
3 non-target plant species	MITC	Seedling emergence test	ER_{50} NOER	14.2 mg MITC/kg dw soil 5.42 mg MITC/kg dw soil	CA8.6.2/02 [redacted] 2008
10 non-target plant species	MITC	Seedling emergence test	ER_{50} NOER	**4.32 kg MITC/ha (equivalent to 5.76 mg MITC/kg dw soil)** 0.840 kg MITC/ha (equivalent to 1.12 mg MITC/kg dw soil)	CA8.6.2/03 [redacted], 2017
6 non-target plant species	DMTU	Seedling emergence test	ER_{50} NOER	6.29 mg DMTU/kg dw soil < 0.9 mg DMTU/kg dw soil	CA8.6.2/04 [redacted], 2011

*Notes: **bold** – values used in the risk assessment; [a]only the lowest value for any of the tested species is shown*

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No specific information was submitted.

2.9.8 Summary of effects on biological methods for sewage treatment

The inhibitory effect of the main metabolite MITC on the oxygen consumption of activated sludge suspension (1.6 g sludge (dry matter)/L water in the incubation vessels) was determined. The lowest measured endpoints were those for nitrification respiration after 3 hours of exposure. The 3-hour EC_{50} for nitrification was 0.990 mg MITC/L, based on initial measured concentrations, and 0.311 mg MITC/L based on mean measured concentrations.

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Birds

The risk assessment included in Volume 3 (PPP) sections B.9.2.1 indicated an acceptable risk to birds for the proposed uses of Metam Sodium 51% (both indoor and outdoor uses - Notifier: Lainco) and Metam Na 510 SL (indoor – Notifier: Taminco)

The risk assessment for effects on birds is conducted in accordance with Regulation (EC) No. 1107/2009 and is normally based on the latest **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**[28]. However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam was performed as explained below.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

Table 2.9.9.1-1: Summary of avian toxicity data used in the risk assessment for metam/MITC

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus)*	Metam-sodium	Single dose (Acute oral toxicity)	Oral LD_{50}	211 mg a.s./kg bw (equivalent to **119 mg MITC/kg bw**)	CA8.1.1.1/01 [redacted] 1985
Bobwhite quail (*Colinus virginianus*)	MITC	Single dose (Acute whole-body inhalation toxicity)	Inhalation LC_{50}	**127 ppm MITC (males)** 181 ppm MITC (females)	CA8.1.1.1/02 [redacted], 2012

*Note: **bold** – endpoint used for the current risk assessment*

Metam Sodium 51% SL (notifier: Lainco)

According to the EFSA Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[29], a risk assessment for birds is not required for applications in **permanent greenhouses**. Also, it is proposed that a risk assessment for birds is conducted for applications in **walk-in tunnels** since exposure to birds is likely. However, in this case of a soil fumigant the walk-in tunnels will be closed at the moment of application in order to be most effective. RMS considers that the potential exposure of birds to MITC outside the walk-in tunnel will be limited in time and therefore the scenario of permanent greenhouses is also appropriate to address the risk.
However, even for permanent greenhouses, exposure is possible since the active metabolite MITC is a highly volatile molecule, and will be present in both the liquid and the gas phase of the soil after its formation from metam. Following volatilisation from the soil, the MITC vapours can leave the greenhouse through the vents, and contaminate the off-field area around the greenhouse. Therefore, **birds can potentially be exposed to vapours of MITC.**
Also, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the treated greenhouse. Consequently, **birds can be exposed to these MITC deposits off-field**.
Finally, birds can be exposed following the **outdoor field use** of metam applied at 153 kg a.s./ha (86.6 kg MITC/ha) by inhalation or dietary consumption of contaminated food items.

Risk assessment for birds exposed to MITC through inhalation:

The **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** does not contain any specific methodology to assess the risk to birds from the inhalation route. Consequently, the former guidance

[28] European Food Safety Authority, 2009. Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. 139 pp.
[29] EFSA (European Food Safety Authority) (2015). Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp.

document 'Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC' (SANCO/4145/2000) is used.

As a worst-case, conservative exposure levels via the inhalation route have been estimated based on EPPO (1994)[30] mentioned in the former 'Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC' (SANCO/4145/2000):

Predicted maximum inhalation dose (µg/kg bw/day) = PEC_{air} (µg/m³) x inhalation rate (m³/day) / W

For non-passerine birds: inhalation rate (m³/day) = 0.4089 $W^{0.77}$
Where W = body weight in kg

The highest measured off-field MITC concentration in air for the proposed **indoor use** through drip irrigation was 292 µg MITC/m³ (see Volume 3 B.9 CP Lainco). The exposure value is worst-case since the application rate in the study (612 kg metam/ha) was higher than the intended use (306 kg metam/ha).

Assuming a bird with body weight of 100 g:

Inhalation rate = 0.4089 x $(0.1)^{0.77}$ = 0.0694 m³/day

Predicted maximum inhalation dose = 292 µg MITC/m³ x 0.0694 / 0.1 m³/kg bw/day
= 203 µg MITC/kg bw/day

The relevant inhalation LC_{50} value for birds following acute whole-body inhalation exposure is 127 ppm (see in Volume 3 B.9 CP Lainco).

Based on the above predicted maximum inhalation dose (203 µg MITC/kg bw/day) and the relevant inhalation LC_{50} value (127000 µg MITC/kg bw), the risk is calculated:

Ratio of daily intake / LC_{50} = 0.0016

Based on Note 1 of EPPO (1994) a low risk to birds via inhalation exposure is therefore identified as this daily intake/LC_{50} ratio is ≤ 0.01.

For completeness, an inhalation TER is also calculated:

$TER_{A,\ inhalation}$ = 127000 / 203 = 626

This acute TER for inhalation is well above the trigger of 10 used to conclude on a low risk for acute avian risk assessments in the EFSA Guidance on Risk Assessment for Birds and Mammals (2009) for the **indoor use** (permanent greenhouse and walk-in tunnel with application of 306 kg metam/ha, respectively 173.2 kg MITC/ha).

The highest measured off-field MITC concentration in air for the proposed **outdoor use** through soil injection was 536 µg MITC/m³ (see Volume 3 B.9 CP Lainco). The exposure value is worst-case since the application method did not make use of the TIF film, instead the soil was compacted with a roller.

Assuming a bird with body weight of 100 g:

Inhalation rate = 0.4089 x $(0.1)^{0.77}$ = 0.0694 m³/day

[30] EPPO (1994). Decision-making scheme for the environmental risk assessment of plant protection products. Chapter 11: Terrestrial vertebrates. OEPP/EPPO Bulletin 24, 37-87.

Predicted maximum inhalation dose = 536 µg MITC/m³ x 0.0694 / 0.1 m³/kg bw/day
= 372 µg MITC/kg bw/day

The relevant inhalation LC_{50} value for birds following acute whole-body inhalation exposure is 127 ppm (see in Volume 3 B.9 CP Lainco).

Based on the above predicted maximum inhalation dose (372 µg MITC/kg bw/day) and the relevant inhalation LC_{50} value (127000 µg MITC/kg bw), the risk is calculated:

Ratio of daily intake / LC_{50} = 0.0029

Based on Note 1 of EPPO (1994) a low risk to birds via inhalation exposure is therefore identified as this daily intake/LC_{50} ratio is ≤ 0.01.

For completeness, an inhalation TER is also calculated:

$TER_{A,\ inhalation}$ = 127000 / 372 = 341

This acute TER for inhalation is well above the trigger of 10 used to conclude on a low risk for acute avian risk assessments in the EFSA Guidance on Risk Assessment for Birds and Mammals (2009) for the **outdoor use** (application of 153 kg metam/ha, respectively 86.6 kg MITC/ha).

As the TIF is an effective mitigation measure to prevent significant volatilisation of MITC to air (see also the study by [redacted], 2019; refer to Volume 3 (CA) Section B.8.3.2 for a summary), the potential exposure of birds to MITC vapour is very low during the first 21 days after application when the soil is covered with TIF. Taking into account the worst case soil DT_{50} of MITC of 3.24 days (at 153 kg metam sodium/ha) or 5 days (at 306 kg metam sodium/ha) (please refer to Volume 3 (CP) Section B.8.1.1 for details), the amount of MITC that is still left in the soil at the moment of TIF removal (21 days after application) will also be low. After removal of the TIF, the soil will be superficially re-worked. This will result in only a short-term aeration of any remaining MITC, which is expected to rapidly dissipate. Consequently, a significant vapour exposure to birds is therefore rather unlikely after removal of the TIF.

Risk assessment for birds exposed to MITC through deposition after volatilisation (off-field)

Acute dietary risk assessment for indoor use (306 kg metam/ha, 173.2 kg MITC/ha)

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 6 in EFSA GD). The indicator species is the small omnivorous bird with the shortcut value of 160.3. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value

The toxicity-exposure ratio is calculated:

$TER_A = LD_{50}$ / DDD

Table 2.9.9.1-2: Acute dietary risk assessment for birds exposed to MITC after volatilisation and deposition in surrounding areas following indoor use of metam

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small omnivorous bird	160.3	0.00292	0.468	119	254

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for birds potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following indoor use of metam.

Acute dietary risk assessment for outdoor use (153 kg metam/ha, 86.6 kg MITC/ha)

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 6 in EFSA GD). The indicator species is the small omnivorous bird with the shortcut value of 160.3. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$$DDD_{\text{single application}} = \text{application rate [kg/ha]} \times \text{shortcut value}$$

The toxicity-exposure ratio is calculated:

$$TER_A = LD_{50} / DDD$$

Table 2.9.9.1-3: Acute dietary risk assessment for birds exposed to MITC after volatilisation and deposition in surrounding areas following outdoor use of metam

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small omnivorous bird	160.3	0.00536	0.859	119	138

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for birds potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following outdoor use of metam.

For completeness, the acute risk for birds exposed to highly contaminated food items (insects, earthworms exposed to the application rate) is also calculated. This assumes the extreme worst-case that soil invertebrates escape from the covering TIF film or that birds are picking directly food items through the TIF film.

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), the bare soil is chosen as the relevant scenario. The indicator species selected are a small granivorous bird ‘finch’, a small

omnivorous bird 'lark' and a small insectivorous bird 'wagtail'. The acute risk from dietary consumption based on the standard residue values is presented below. It should however be noted that this guidance is more applicable to spray applications and the likelihood of dietary exposure at the application rate is considered minimal.

Table 2.9.9.1-4: Acute dietary risk assessment for birds exposed to highly contaminated food items at the application rate for outdoor use of metam

Crop	Indicator species	Shortcut value for acute assessment (90% RUD)	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Bare soil (BBCH < 10)	Small granivorous bird 'finch' 100 % weed seeds	24.7	86.6	2139	119	0.056
Bare soil (BBCH < 10)	Small omnivorous bird 'lark' 100 % soil dwelling invertebrates	9.3	86.6	805	119	0.148
	Small omnivorous bird 'lark' 100 % seeds	20.2	86.6	1749	119	0.068
	Small omnivorous bird 'lark' 50 % seeds and 50 % ground arthropods	17.4	86.6	1507	119	0.079
Bare soil (BBCH < 10)	Small insectivorous bird 'wagtail' 100 % soil dwelling invertebrates	10.9	86.6	944	119	0.126

The acute TER values do not exceed the trigger value of 10. Refinement of the calculations is based on the measured residues in the food items (see Volume 3 B.9 CP Lainco). For the small granivorous bird 'finch' the refined RUD value was based on the maximum MITC levels found in seeds ([redacted], 2014). For the small omnivorous bird 'lark' the refined RUD value was based on a mixed diet of 50 % weed seeds and 50 % ground arthropods; the RUD value was calculated as an average of maximum RUD value for seeds (0.007 mg MITC/kg; [redacted], 2014) and average maximum RUD value for ground arthropods (0.436 mg MITC/kg; [redacted] 2014b and [redacted] 2014). For small insectivorous bird 'wagtail' the refined RUD value was calculated as an average of maximum RUD value for ground arthropods (0.436 mg MITC/kg; [redacted], 2014b and [redacted], 2014) and earthworms (0.397 mg MITC/kg; [redacted]2014a and [redacted] 2014).

Table 2.9.9.1-5: Acute dietary risk assessment for birds exposed to highly contaminated food items at the application rate for outdoor use of metam based on measured residue data

Crop	Indicator species	FIR/bw	Refined RUD	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Bare soil (BBCH < 10)	Small granivorous bird 'finch' 100 % weed seeds	0.28	0.007	0.170	119	701
Bare soil (BBCH < 10)	Small omnivorous bird 'lark' 50 % seeds and 50 % ground arthropods	0.35	0.222	6.73	119	18
Bare soil (BBCH < 10)	Small insectivorous bird 'wagtail' 100 % soil dwelling invertebrates	0.79	0.417	28.5	119	4.2

The acute TER exceed trigger value of 10 for small granivorous birds and small omnivorous birds. Further refinement is needed for small insectivorous birds. Refinement of the calculations is based on the measured residues in the food items (see in Volume 3 B.9 CP Lainco). The refined RUD value is based on a diet of 100 % soil dwelling invertebrates; the RUD value was calculated as an average of 90th percentile RUD values from measured residues 7-10 days after application for ground arthropods and earthworms.

Table 2.9.9.1-6: Acute dietary risk assessment for birds exposed to highly contaminated food items at the application rate for outdoor use of metam based on measured residue data

Crop	Indicator species	FIR/bw	Refined RUD	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Bare soil (BBCH < 10)	Small insectivorous bird 'wagtail' 100 % soil dwelling invertebrates	0.79	0.124	8.48	119	14

In conclusion, the acute risk for birds accidentally exposed to highly contaminated food items following the outdoor use of metam is considered acceptable based on measured residue data.

Reproductive dietary risk assessment

No reproductive toxicity data is available for birds. In the interests of reducing vertebrate testing, no reproductive data have been generated. Reference to the acute risk assessment, however, suggests extremely low risk to birds. In addition, long-term exposure is considered unlikely due to the rapid conversion of metam to MITC and MITC itself.

Risk assessment of the consumption of drinking water

The EFSA Guidance on Risk Assessment for Birds and Mammals (2009) states that due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by birds, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{OC} ≥ 500 L/kg).

For MITC the K_{OC} value is 21.7 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour) and the effective application rate of 5.36 g MITC/ha via volatilisation and deposition form outdoor use (covering for the 2.92 g MITC/ha related to indoor use). The acute LD_{50} value is 119 mg MITC/kg bw.
The ratios of effective application rate to the relevant endpoint for the intended uses of Metam 510 g/L SL are shown below.

Table 2.9.9.1-7: Ratios of effective application rate to endpoints for birds exposed to MITC after volatilisation and deposition in surrounding areas following outdoor use of metam

Intended use	AR_{eff} (g MITC/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	Ratio trigger
Outdoor use	5.36	119	22	50

The resulting ratio is clearly below the trigger value of 50 indicating that the **acute risk to birds via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculation.

Effects of secondary poisoning

The log P_{OW} of metam-sodium amounts to ≤ -2.91 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of MITC amounts to 1.05 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of DMTU amounts to ≤ -0.20 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
In conclusion, a risk assessment for effects due to secondary poisoning is therefore not required.

Metam Na 510 SL (Notifier: Taminco)

According to the EFSA Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[31], a risk assessment for birds is not required for applications in **permanent greenhouses**.
However, the active metabolite MITC is a highly volatile molecule, and will be present in both the liquid and the gas phase of the soil after its formation from metam. Following volatilisation from the soil, the MITC vapours can leave the greenhouse through the vents, and contaminate the off-field area around the greenhouse. Therefore, **birds can potentially be exposed to vapours of MITC.**
Finally, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the treated greenhouse. Consequently, **birds can be exposed to these MITC deposits off-field**.

Risk assessment for birds exposed to MITC through inhalation

The **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** does not contain any specific methodology to assess the risk to birds from the inhalation route. Consequently, the former guidance document 'Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC' (SANCO/4145/2000) is used.

[31] EFSA (European Food Safety Authority) (2015). Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp.

As a worst-case, conservative exposure levels via the inhalation route have been estimated based on EPPO (1994)[32] mentioned in the former 'Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC' (SANCO/4145/2000):

Predicted maximum inhalation dose (µg/kg bw/day) = PEC_{air} (µg/m³) x inhalation rate (m³/day) / W

For non-passerine birds: inhalation rate (m³/day) = 0.4089 $W^{0.77}$
Where W = body weight in kg

The highest measured off-field MITC concentration in air for the proposed indoor use through drip irrigation was 39.74 µg MITC/m³ (see Volume 3 B.9 CP Taminco).

Assuming a bird with body weight of 100 g:

Inhalation rate = 0.4089 x $(0.1)^{0.77}$ = 0.0694 m³/day

Predicted maximum inhalation dose = 39.74 µg MITC/m³ x 0.0694 / 0.1 m³/kg bw/day
= 27.58 µg MITC/kg bw/day

The relevant inhalation LC_{50} value for birds following acute whole-body inhalation exposure is 127 ppm (see Volume 3 B.9 CP Taminco).

Based on the above predicted maximum inhalation dose (27.58 µg MITC/kg bw/day) and the relevant inhalation LC_{50} value (127000 µg MITC/kg bw), the risk is calculated:

Ratio of daily intake / LC_{50} = 0.000217

Based on Note 1 of EPPO (1994) a low risk to birds via inhalation exposure is therefore identified as this daily intake/LC_{50} ratio is ≤ 0.01.

For completeness, an inhalation TER is also calculated:

$TER_{A,\ inhalation}$ = 127000 / 27.58 = 4605

This acute TER for inhalation is well above the trigger of 10 used to conclude on a low risk for acute avian risk assessments in the EFSA Guidance on Risk Assessment for Birds and Mammals (2009).

Risk assessment for birds exposed to MITC through deposition after volatilisation (off-field)

Acute dietary risk assessment

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 6 in EFSA GD). The indicator species is the small omnivorous bird with the shortcut value of 160.3. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food

[32] EPPO (1994). Decision-making scheme for the environmental risk assessment of plant protection products. Chapter 11: Terrestrial vertebrates. OEPP/EPPO Bulletin 24, 37-87.

intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value

The toxicity-exposure ratio is calculated:

$TER_A = LD_{50} / DDD$

Table 2.9.9.1-8: Acute dietary risk assessment for birds exposed to MITC after volatilisation and deposition in surrounding areas following use of metam in permanent greenhouses

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small omnivorous bird	160.3	0.00084	0.135	119	884

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for birds potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following use of metam in permanent greenhouses.

Reproductive dietary risk assessment

No reproductive toxicity data is available for birds. In the interests of reducing vertebrate testing, no reproductive data have been generated. Reference to the acute risk assessment, however, suggests extremely low risk to birds. In addition, long-term exposure is considered unlikely due to the rapid conversion of metam to MITC and MITC itself.

Risk assessment of the consumption of drinking water

The EFSA Guidance on Risk Assessment for Birds and Mammals (2009) states that due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by birds, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances ($K_{OC} \geq$ 500 L/kg).

For MITC the K_{OC} value is 21.7 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour) and the effective application rate of 0.84 g MITC/ha via volatilisation and deposition outside the permanent greenhouses where metam is applied. The acute LD_{50} value is 119 mg MITC/kg bw.
The ratios of effective application rate to the relevant endpoint for the intended uses of Metam 510 g/L SL are shown below.

Table 2.9.9.1-9: Ratios of effective application rate to endpoints for birds exposed to MITC after volatilisation and deposition in surrounding areas following use of metam in permanent greenhouses

Intended use	AR_{eff} (g MITC/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	Ratio trigger
Permanent greenhouses	0.84	119	0.007	50

The resulting ratio is clearly below the trigger value of 50 indicating that the **acute risk to birds via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculation.

Effects of secondary poisoning

The log P_{OW} of metam-sodium amounts to ≤ -2.91 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of MITC amounts to 1.05 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of DMTU amounts to ≤ -0.20 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
In conclusion, a risk assessment for effects due to secondary poisoning is therefore not required.

2.9.9.2 Mammals

The risk assessment included in Volume 3 (PPP) sections B.9.2.2 indicated an acceptable risk to mammals for the proposed uses of Metam Sodium 51% (both indoor and outdoor uses - Notifier: Lainco) and Metam Na 510 SL (indoor – Notifier: Taminco)

The risk assessment for effects on mammals is conducted in accordance with Regulation (EC) No. 1107/2009 and is normally based on the latest **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**[33]. However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam was performed as explained below.
The potential risk from DMTU is considered covered by the risk assessment for MITC.

Table 2.9.9.2-1: Summary of mammalian toxicity data used in the risk assessment for metam/MITC

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	MITC	Single dose (Acute oral toxicity)	Oral LD_{50}	**100 mg/kg bw**	EFSA Journal 2011; 9(9):2334*
Rat	MITC	Single dose (Acute whole-body inhalation toxicity)	Inhalation LC_{50}	**0.54 mg/L (air) (4 hour)**	[redacted], 1981
Rat	MITC	28 day (Subchronic inhalation toxicity)	NOAEL	**5 mg/m³ (air)** (1.35 mg/kg bw/day)	[redacted], 1987
Rat	MITC	2-year oral (drinking water) toxicity and carcinogenicity study	NOAEL	**0.44 mg/kg bw/day**	[redacted], 1984

<u>Metam Sodium 51% SL (notifier: Lainco)</u>

[33] European Food Safety Authority, 2009. Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. 139 pp.

According to the EFSA Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[34], a risk assessment for mammals is not required for applications in **permanent greenhouses**. Also, it is proposed that a risk assessment for mammals is conducted for applications in **walk-in tunnels** since exposure to mammals is likely. However, in this case of a soil fumigant the walk-in tunnels will be closed at the moment of application in order to be most effective. RMS considers that the potential exposure of mammals to MITC outside the walk-in tunnel will be limited in time and therefore the scenario of permanent greenhouses is also appropriate to address the risk.
However, even for permanent greenhouses, exposure is possible since the active metabolite MITC is a highly volatile molecule, and will be present in both the liquid and the gas phase of the soil after its formation from metam. Following volatilisation from the soil, the MITC vapours can leave the greenhouse through the vents, and contaminate the off-field area around the greenhouse. Therefore, **mammals can potentially be exposed to vapours of MITC.**
Also, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the treated greenhouse. Consequently, **mammals can be exposed to these MITC deposits off-field**.
Finally, mammals can be exposed following the **outdoor field use** of metam applied at 153 kg a.s./ha (86.6 kg MITC/ha) by inhalation or dietary consumption of contaminated food items.

Risk assessment for mammals exposed to MITC through inhalation

The **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** does not contain any specific methodology to assess the risk to mammals from the inhalation route.

Small mammals may be exposed to the airborne fraction of MITC *via* air following volatilization.

Acute (4 hours exposure) and short-term (28 days exposure) effect endpoints are available:

LC_{50} (4 h) = 0.54 mg MITC/L air = 540 mg MITC/m³ air
NOAEL (28 d) = 5 mg MITC/m³ air

The acute and short-term effect endpoints expressed as concentrations in air can be directly compared to the estimated concentration of MITC in air following indoor and outdoor use.

Table 2.9.9.2-2: Acute mammalian inhalation risk assessment for MITC exposure

Scenario	Exposure duration	Exposure (air concentration, mg MITC/m³)	LD_{50} (mg MITC/m³ air)	TER acute inhalation	Trigger
Exposure to MITC *via* air: indoor use	Acute (4 h)	0.292	540	1849	10
Exposure to MITC *via* air: outdoor use		0.536	540	1007	10

Table 2.9.9.2-3: Short-term mammalian inhalation risk assessment for MITC exposure

Scenario	Exposure duration	Exposure (air concentration, mg MITC/m³)	NOAEL (mg MITC/m³ air)	TER short-term inhalation	Trigger
Exposure to MITC *via* air: indoor use	Short-term (28 d)	0.292	5	17	10

[34] EFSA (European Food Safety Authority) (2015). Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp.

Exposure to MITC *via* air: outdoor use		0.536	5	9.3	10

As a weight of evidence, the trigger value was set at 10, in comparison with acute dietary risk assessment. Consequently, the risk assessment for acute and short-term inhalation indicate acceptable risk to mammals from exposure to MITC *via* inhalation. It is to be noted that the risk assessment assumes constant exposure to maximal levels of MITC *via* air. Particularly for the short-term risk assessment (with reference to a 28-day exposure study) this is unlikely. Moreover, the exposure estimates used in the risk calculations were worst-case (higher application rate and no use of covering TIF film).

As the TIF is an effective mitigation measure to prevent significant volatilisation of MITC to air (see also the study by [redacted], 2019; refer to Volume 3 (CA) Section B.8.3.2 for a summary), the potential exposure of mammals to MITC vapour is very low during the first 21 days after application when the soil is covered with TIF. Taking into account the worst case soil DT_{50} of MITC of 3.24 days (at 153 kg metam sodium/ha) or 5 days (at 306 kg metam sodium/ha) (please refer to Volume 3 (CP) Section B.8.1.1 for details), the amount of MITC that is still left in the soil at the moment of TIF removal (21 days after application) will also be low. After removal of the TIF, the soil will be superficially re-worked. This will result in only a short-term aeration of any remaining MITC, which is expected to rapidly dissipate. Consequently, a significant vapour exposure to mammals is therefore rather unlikely after removal of the TIF.

Risk assessment for mammals exposed to MITC through deposition after volatilisation (off-field)

Acute dietary risk assessment for indoor use (306 kg metam/ha, 173.2 kg MITC/ha)

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 8 in EFSA GD). The indicator species is the small herbivorous mammal with the shortcut value of 136.4. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value

The toxicity-exposure ratio is calculated:

$TER_A = LD_{50} / DDD$

Table 2.9.9.2-4: Acute dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following indoor use of metam

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small herbivorous mammal	136.4	0.00292	0.398	100	251

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for mammals potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following indoor use of metam.

Acute dietary risk assessment for outdoor use (153 kg metam/ha, 86.6 kg MITC/ha)

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 8 in EFSA GD). The indicator species is the small herbivorous mammal with the shortcut value of 136.4. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value

The toxicity-exposure ratio is calculated:

$TER_A = LD_{50} / DDD$

Table 2.9.9.2-5: Acute dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following outdoor use of metam

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small herbivorous mammal	136.4	0.00536	0.731	100	137

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for mammals potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following outdoor use of metam.

For completeness, the acute risk for mammals exposed to highly contaminated food items (insects, earthworms exposed to the application rate) is also calculated. This assumes the extreme worst-case that soil invertebrates escape from the covering TIF film or that mammals are picking directly food items through the TIF film.

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), the bare soil is chosen as the relevant scenario. The indicator species selected is a small omnivorous mammal ‘mouse’, represented by the wood mouse (*Apodemus sylvaticus*). The acute risk from dietary consumption based on the standard residue values is presented below. It should however be noted that this guidance is more applicable to spray applications and the likelihood of dietary exposure at the application rate is considered minimal.

Table 2.9.9.2-6: Acute dietary risk assessment for mammals exposed to highly contaminated food items at the application rate for outdoor use of metam

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A

		(90% RUD)				
Bare soil (BBCH < 10)	Small omnivorous mammal 'wood mouse' 50 % weed seeds and 50 % ground arthropods	14.3	86.6	1238	100	0.081

The acute TER values does not exceed the trigger value of 10. Refinement of the calculations is based on the measured residues in the food items (see in Volume 3 B.9 CP Taminco). For the small omnivorous mammal 'wood mouse' the refined RUD value was based on a mixed diet of 50 % weed seeds and 50 % ground arthropods; the RUD value was calculated as an average of maximum RUD value for seeds (0.007 mg MITC/kg; [redacted], 2014) and average maximum RUD value for ground arthropods (0.436 mg MITC/kg; [redacted], 20104b and [redacted], 2014).

Table 2.9.9.2-7: Acute dietary risk assessment for mammals exposed to highly contaminated food items at the application rate for outdoor use of metam based on measured residue data

Crop	**Indicator species**	**FIR/bw**	**Refined RUD**	**DDD (mg a.s./kg bw/day)**	**LD_{50} (mg a.s./kg bw)**	**TER_A**
Bare soil (BBCH < 10)	Small omnivorous mammal 'wood mouse' 50 % weed seeds and 50 % ground arthropods	0.24	0.222	4.61	100	22

The acute TER exceed trigger value of 10 for small herbivorous mammals. In conclusion, the acute risk for mammals accidentally exposed to highly contaminated food items following the outdoor use of metam is considered acceptable based on measured residue data.

Reproductive dietary risk assessment

The reproductive dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for reproductive risk assessment was chosen, e.g. the scenario for cotton (refer to Table 12 in EFSA GD). The indicator species is the small herbivorous mammal with the shortcut value of 72.3. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value x TWA

Since the toxic effect is considered to be caused by a long-term exposure, the TWA is set at 0.53 (estimates time-weighed exposure over 21 days, assuming a default DT_{50} 10 days).

The toxicity-exposure ratio is calculated:

TER_{LT} = NOAEL / DDD

Table 2.9.9.2-8: Reproductive dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following indoor use of metam

Crop	Indicator species	Shortcut value for reproductive assessment	Application rate (kg MITC/ha)	TWA	DDD (mg a.s./kg bw/day)	NOAEL (mg a.s./kg bw/day)	TER_{LT}
Cotton	Small herbivorous mammal	72.3	0.00292	0.53	0.112	0.44	3.9

Table 2.9.9.2-9: Reproductive dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following outdoor use of metam

Crop	Indicator species	Shortcut value for reproductive assessment	Application rate (kg MITC/ha)	TWA	DDD (mg a.s./kg bw/day)	NOAEL (mg a.s./kg bw/day)	TER_{LT}
Cotton	Small herbivorous mammal	72.3	0.00536	0.53	0.205	0.44	2.1

The long-term TER do not exceed the trigger value of 5. However, the dietary calculations are not appropriate to the intended use of a soil fumigant. Moreover, the exposure to MITC is an overestimation already in acute risk calculation, which is even more the case in addressing long-term risk. The calculations for the reproductive risk were presented for completeness, however it is the opinion of the RMS that no further refinement of the TER calculations are needed since it can be reasonably concluded that risk is considered acceptable.

Risk assessment of the consumption of drinking water

The EFSA Guidance on Risk Assessment for Birds and Mammals (2009) states that due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by mammals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{OC} ≥ 500 L/kg).

For MITC the K_{OC} value is 21.7 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour) and the effective application rate of 5.36 g MITC/ha via volatilisation and deposition form outdoor use (covering for the 2.92 g MITC/ha related to indoor use). The acute LD_{50} value is 100 mg MITC/kg bw and the NOAEL value is 0.44 mg MITC/kg bw/day.
The ratios of effective application rate to the relevant endpoint for the intended uses of Metam 510 g/L SL are shown below.

Table 2.9.9.2-10: Ratios of effective application rate to endpoints for mammals exposed to MITC after volatilisation and deposition in surrounding areas following outdoor use of metam

Intended use	AR_{eff} (g MITC/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOAEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOAEL	Ratio trigger
Outdoor use	5.36	100	0.0536	0.44	12	50

The resulting ratios are clearly below the trigger value of 50 indicating that the **acute and long-term risk to mammals via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculation.

Effects of secondary poisoning

The log P_{OW} of metam-sodium amounts to ≤ -2.91 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of MITC amounts to 1.05 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of DMTU amounts to ≤ -0.20 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
In conclusion, a risk assessment for effects due to secondary poisoning is therefore not required.

Metam Na 510 SL (Notifier: Taminco)

According to the EFSA Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[35], a risk assessment for mammals is not required for applications in **permanent greenhouses**.
However, the active metabolite MITC is a highly volatile molecule, and will be present in both the liquid and the gas phase of the soil after its formation from metam. Following volatilisation from the soil, the MITC vapours can leave the greenhouse through the vents, and contaminate the off-field area around the greenhouse. Therefore, **mammals can potentially be exposed to vapours of MITC.**
Finally, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the treated greenhouse. Consequently, **mammals can be exposed to these MITC deposits off-field**.

Risk assessment for mammals exposed to MITC through inhalation

The **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** does not contain any specific methodology to assess the risk to mammals from the inhalation route.

Small mammals may be exposed to the airborne fraction of MITC *via* air following volatilization. The estimated exposure concentration is 39.74 µg MITC/m³ air.

Acute (4 hours exposure) and short-term (28 days exposure) effect endpoints are available:

LC_{50} (4 h) = 0.54 mg MITC/L air = 540 mg MITC/m³ air
NOAEL (28 d) = 5 mg MITC/m³ air

The acute and short-term effect endpoints expressed as concentrations in air can be directly compared to the estimated concentration of MITC in air (0.03974 mg/m³) following drip irrigation to soil in permanent greenhouses.

Table 2.9.9.2-11: Acute mammalian inhalation risk assessment for MITC exposure

[35] EFSA (European Food Safety Authority) (2015). Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp.

Scenario	Exposure duration	Exposure (air concentration, mg MITC/m³)	LD_{50} (mg MITC/m³ air)	TER acute inhalation	Trigger
Exposure to MITC *via* air	Acute (4 h)	0.03974	540	13588	10

Table 2.9.9.2-12: Short-term mammalian inhalation risk assessment for MITC exposure

Scenario	Exposure duration	Exposure (air concentration, mg MITC/m³)	NOAEL (mg MITC/m³ air)	TER short-term inhalation	Trigger
Exposure to MITC *via* air	Short-term (28 d)	0.03974	5	126	10

As a weight of evidence, the trigger value was set at 10, in comparison with acute dietary risk assessment. Consequently, the risk assessment for acute and short-term inhalation indicate acceptable risk to mammals from exposure to MITC *via* inhalation. It is to be noted that the risk assessment assumes constant exposure to maximal levels of MITC *via* air. Particularly for the short-term risk assessment (with reference to a 28-day exposure study) this is unlikely.

Risk assessment for mammals exposed to MITC through deposition after volatilisation (off-field)

Acute dietary risk assessment

The acute dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for acute risk assessment was chosen, e.g. the scenario for cotton (refer to Table 8 in EFSA GD). The indicator species is the small herbivorous mammal with the shortcut value of 136.4. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value

The toxicity-exposure ratio is calculated:

$TER_A = LD_{50} / DDD$

Table 2.9.9.2-13: Acute dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following use of metam in permanent greenhouses

Crop	Indicator species	Shortcut value for acute assessment	Application rate (kg MITC/ha)	DDD (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Cotton	Small herbivorous mammal	136.4	0.00084	0.115	100	873

The acute TER exceeds largely the trigger value of 10, indicating **acceptable risk for mammals potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following use of metam in permanent greenhouses.

Reproductive dietary risk assessment

The reproductive dietary risk assessment is conducted according to the procedures outlined in EFSA Guidance on Risk Assessment for Birds and Mammals (2009), starting with the screening step. The screening assessment requires the identification of the indicator species relevant to the crop off-field the permanent greenhouse where metam was applied. In order to cover the worst-case situation, the highest short-cut value for reproductive risk assessment was chosen, e.g. the scenario for cotton (refer to Table 12 in EFSA GD). The indicator species is the small herbivorous mammal with the shortcut value of 72.3. While cotton is unlikely to be grown in the areas surrounding the permanent greenhouses in which metam is applied, this represents the worst-case in terms of residues per unit dose and food intake rate for the relevant indicator species (RUD and FIR/bw are integrated into the short-cut value). This is combined with the application rate (maximum deposition rate of MITC in kg/ha) to estimate exposure.

The daily dietary dose (DDD) for a single application is calculated:

$DDD_{single\ application}$ = application rate [kg/ha] x shortcut value x TWA

Since the toxic effect is considered to be caused by a long-term exposure, the TWA is set at 0.53 (estimates time-weighed exposure over 21 days, assuming a default DT_{50} 10 days).

The toxicity-exposure ratio is calculated:

TER_{LT} = NOAEL / DDD

Table 2.9.9.2-14: Reproductive dietary risk assessment for mammals exposed to MITC after volatilisation and deposition in surrounding areas following use of metam in permanent greenhouses

Crop	Indicator species	Shortcut value for reproductive assessment	Application rate (kg MITC/ha)	TWA	DDD (mg a.s./kg bw/day)	NOAEL (mg a.s./kg bw/day)	TER_{LT}
Cotton	Small herbivorous mammal	72.3	0.00084	0.53	0.0322	0.44	14

The long-term TER exceeds largely the trigger value of 5, indicating **acceptable risk for mammals potentially exposed through dietary intake of MITC after volatilisation and redeposition** in surrounding areas following use of metam in permanent greenhouses.

Risk assessment of the consumption of drinking water

The EFSA Guidance on Risk Assessment for Birds and Mammals (2009) states that due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by mammals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{OC} ≥ 500 L/kg).

For MITC the K_{OC} value is 21.7 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour) and the effective application rate of 0.84 g MITC/ha via volatilisation and deposition outside the permanent greenhouses where metam is applied. The acute LD_{50} value is 100 mg MITC/kg bw and the NOAEL value is 0.44 mg MITC/kg bw/day.

The ratios of effective application rate to the relevant endpoint for the intended uses of Metam 510 g/L SL are shown below.

Table 2.9.9.2-15: Ratios of effective application rate to endpoints for mammals exposed to MITC after volatilisation and deposition in surrounding areas following use of metam in permanent greenhouses

Intended use	AR_{eff} (g MITC/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOAEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOAEL	Ratio trigger
Permanent greenhouses	0.84	100	0.0084	0.44	1.91	50

The resulting ratios are clearly below the trigger value of 50 indicating that the **acute and long-term risk to mammals via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculation.

Effects of secondary poisoning

The log P_{OW} of metam-sodium amounts to ≤ -2.91 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of MITC amounts to 1.05 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
The log P_{OW} of DMTU amounts to ≤ -0.20 (EFSA Journal 2011; 9(9): 2334) and thus does not exceed the trigger value of 3.
In conclusion, a risk assessment for effects due to secondary poisoning is therefore not required.

2.9.9.3 Aquatic organisms

The risk assessment included in Volume 3 (PPP) sections B.9.4 did not indicate an acceptable risk to aquatic organisms for the proposed uses of Metam Sodium 51% (both indoor and outdoor uses - Notifier: Lainco) and indicated an acceptable risk to aquatic organisms for the proposed uses of Metam Na 510 SL (indoor – Notifier: Taminco)

The risk assessment for effects on aquatic organisms is updated according to the new **EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013)**[36], which has been noted in the meeting of the Standing Committee on Plants, Animals, Food and Feed on 11 July 2014. This document will be referred to as 'the EFSA Guidance Document for aquatic organisms (2013)'.

Table 2.9.9.3-1: Summary of aquatic toxicity data used in the Tier 1 risk assessment for metam/MITC

Substance	Time span	Species group	Test organism	Selected endpoint for use in risk assessment
MITC	Acute	Fish	*Oncorhynchus mykiss*	LC_{50} = 53.1 µg a.s./L
		Aquatic invertebrates	*Daphnia magna*	EC_{50} = 76 µg a.s./L
	Chronic	Fish	*Pimephales promelas*	EC_{10} = 9.24 µg a.s./L
		Aquatic invertebrates	*Daphnia magna*	EC_{10} = 35 µg a.s./L
		Algae	*Pseudokirchneriella subcapitata*	E_rC_{50} = 189 µg a.s./L
		Aquatic plants	*Lemna gibba*	E_rC_{50} = 290 µg a.s./L*

[36] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290. 268 pp.

** Endpoint from study CA8.2.7/03 of the applicant Lainco; applicant Taminco (Eastman) needs an official access to this data*

For the first tier risk assessment, RAC values are determined based on the lowest available endpoints for each group of organisms

Table 2.9.9.3-2: Summary of Tier 1 RAC values used in the risk assessment for metam/MITC

	Species group	Endpoint	Assessment factor	RAC
MITC				
Acute effect assessment	Fish	LC_{50} = 53.1 µg a.s./L	100	0.531 µg a.s./L
	Aquatic invertebrates	EC_{50} = 76 µg a.s./L	100	0.76 µg a.s./L
			Overall acute RAC	**0.531 µg a.s./L**
Chronic effect assessment	Fish	EC_{10} = 9.24 µg a.s./L	10	0.924 µg a.s./L
	Daphnia magna	EC_{10} = 35 µg a.s./L	10	3.5 µg a.s./L
	Algae	E_rC_{50} = 189 µg a.s./L	10	18.9 µg a.s./L
	Aquatic plants	E_rC_{50} = 290 µg a.s./L	10	29.0 µg a.s./L
			Overall chronic RAC	**0.924 µg a.s./L**

Notes: RAC = Regulatory Acceptable Concentration

Metam Sodium 51% SL (notifier: Lainco)

Higher Tier RAC values

For the acute toxicity endpoint of fish, a geometric mean of available acute toxicity studies is presented. According to the EFSA Guidance Document for aquatic organisms (2013), a geometric mean acute toxicity endpoint may be calculated when additional toxicity data are available for <5 vertebrate test species. The standard level of protection is set assuming that a single study with one test species will be available to meet the regulatory data requirement. When data on further test species are available, the uncertainty regarding inter-species sensitivity to the test item decreases. The aquatic guidance confirms that a geometric mean of available additional acute toxicity data (with the Tier 1 assessment factor of 100) preserves the intended level of protection.

Regarding the acute toxicity data for MITC, data on two different test species are available (*Oncorhynchus mykiss* and *Lepomis macrochirus*). For one of these species (*Oncorhynchus mykiss*) two separate studies are available. A geometric mean of these two endpoints for *Oncorhynchus mykiss* has been entered in the overall geometric mean. The calculation of the overall geometric mean is summarised below.

Table 2.9.9.3-3: Tier 2A: Fish acute geometric mean endpoint based on the available acute fish studies for the active metabolite MITC

Reference	Author	Test species	Timescale	LC_{50} (µg a.s./L)		Overall fish acute geometric mean LC_{50} (µg a.s./L)
CA8.2.1./10	[redacted] 2002	*Oncorhynchus mykiss*	96 hours (flow-through)	53.1*	Acute geometric mean for *Oncorhynchus mykiss* LC_{50} = 70 µg a.s./L	100
CA8.2.1/11	[redacted], 1991a	*Oncorhynchus mykiss*	96 hours (flow-through)	94		
CA8.2.1/12	[redacted], 1991b	*Lepomis macrochirus*	96 hours (flow-through)	142		

** Tier 1 endpoint*

The **overall fish acute geometric mean LC_{50} value** for the 3 acute fish studies with 2 different fish species is calculated as **100 µg a.s./L**. In accordance with the procedures outlined in the EFSA Guidance Document for aquatic organisms, an **assessment factor of 100** will be applied to this geomean endpoint in determining the acute RAC value for fish, resulting in **higher tier geomean RAC of 1.0 µg MITC/L**. This RAC value will be considered in the higher tier acute risk assessment for fish.

For **aquatic invertebrates**, additional acute toxicity studies representing a range of species including crustaceans, insects, annelids, molluscs and platyhelminths are performed. According to the EFSA Guidance Document on aquatic organisms (2013) it is considered appropriate to refine the acute aquatic invertebrate toxicity endpoint using the **Tier 2A geomean approach**. The invertebrate acute toxicity data are summarised.

Table 2.9.9.3-4: Tier 2A: Acute geometric mean endpoint based on the available aquatic invertebrate studies for the active metabolite MITC

Reference Author	Test species	Taxonomic grouping	Exposure duration (hours)	EC_{50} (µg a.s./L)	Geometric mean species EC_{50} (µg a.s./L)
CA8.2.4.1/04 [redacted], 2002	*Daphnia magna*	Cladoceran crustacean	48 h (semi-static)	76*	97
CA8.2.4.1/06 [redacted], 2019a			48 h (flow-through)	124	
CA8.2.4.2/05 [redacted] 2014a	*Chironomus riparius*	Dipteran insect	48 h (semi-static)	90.6	71
CA8.2.4.2/03 [redacted]., 2018a			48 h (semi-static)	55	
CA8.2.4.2/15 [redacted] 2019c	*Dugesia tigrina*	Triclad platyhelminth	96 h (semi-static)	137	-
CA8.2.4.2/13 [redacted], 2019a	*Lumbriculus variegatus*	Lumbricid annelid	96 h (semi-static)	315	254
CA8.2.4.2/17 [redacted], 2014c			48 h (semi-static)	205	
CA8.2.4.2/16 [redacted], 2014b	*Crangonyx pseudogracilis*	Amphipod crustacean	48 h (semi-static)	312	-
CA8.2.4.2/14 [redacted], 2019b	*Potamopyrgus antipodarum*	Gastropod mollusc	96 h (semi-static)	319	-

** Tier 1 endpoint*

The **overall aquatic invertebrate acute geometric mean EC_{50} value** for the 9 acute studies with 6 different aquatic invertebrate species is calculated as **170 µg a.s./L**. In accordance with the procedures outlined in the EFSA Guidance Document for aquatic organisms, an **assessment factor of 100** will be applied to this geomean endpoint in determining the acute RAC value for aquatic invertebrates, resulting in **higher tier geomean RAC of 1.70 µg MITC/L**. This RAC value will be considered in the higher tier acute risk assessment for aquatic invertebrates.

Risk assessment

The risk assessment for aquatic organisms living in surface waters adjacent to treated permanent greenhouses (**indoor use at 306 kg metam/ha**) is presented in Volume 3 B.9 CP Lainco. As a worst-case approach, the acute and chronic aquatic risk assessment are based on the highest PEC_{SW} value (see and in Volume 3 B.9 CP Lainco).

Conclusion:
The acute and chronic risk for all aquatic organisms is not considered acceptable at FOCUS step 1 or 2. Based on preliminary refinement at FOCUS step 3 scenarios D3 ditch (early), D3 ditch (late), D4 pond and D6 ditch are considered acceptable for all of the aquatic organisms. However, RMS notes that the PEC_{SW} values presented are still under discussion in the section on Fate and behaviour. The current PEC_{SW} values are not sufficiently taking into account the main route of exposure, which is by volatilization. Since the exposure values are an underestimation, consequently the ecotox risk assessment is uncertain and no further conclusions can be drawn.

The risk assessment for aquatic organisms living in surface waters adjacent to treated fields (**outdoor use at 153 kg metam/ha**) is presented in Volume 3 B.9 CP Lainco. As a worst-case approach, the acute and chronic aquatic risk assessment are based on the highest PEC_{SW} value (see , and in Volume 3 B.9 CP Lainco).

Conclusion:
The acute and chronic risk for all aquatic organisms is not considered acceptable at FOCUS step 1 or 2. Based on preliminary refinement at FOCUS step 3 scenarios R1 stream (1), R3 stream (1 and 2), R4 stream (1 and 2) are not considered acceptable for all of the aquatic organisms. However, RMS notes that the PEC_{SW} values presented are still under discussion in the section on Fate and behaviour. The current PEC_{SW} values are not sufficiently taking into account the main route of exposure, which is by volatilization. Since the exposure values are an underestimation, consequently the ecotox risk assessment is uncertain and no further conclusions can be drawn.

Metam Na 510 SL (Notifier: Taminco)

Higher Tier RAC values

For the acute toxicity endpoint of **fish**, a **geometric mean** of available acute toxicity studies is presented. According to the EFSA Guidance Document for aquatic organisms (2013), a geometric mean acute toxicity endpoint may be calculated when additional toxicity data are available for <5 vertebrate test species. The standard level of protection is set assuming that a single study with one test species will be available to meet the regulatory data requirement. When data on further test species are available, the uncertainty regarding inter-species sensitivity to the test item decreases. The aquatic guidance confirms that a geometric mean of available additional acute toxicity data (with the Tier 1 assessment factor of 100) preserves the intended level of protection.

Regarding the acute toxicity data for MITC, data on three different test species are available (*Oncorhynchus mykiss*, *Lepomis macrochirus* and *Cyprinodon variegatus*). For one of these species (*Oncorhynchus mykiss*) three separate studies are available. A geometric mean of these three endpoints for *Oncorhynchus mykiss* has been entered in the overall geometric mean. The calculation of the overall geometric mean is summarised below.

Table 2.9.9.3-5: Tier 2A: Fish acute geometric mean endpoint based on the available acute fish studies for the active metabolite MITC

Reference	Author	Test species	Timescale	LC_{50} (µg a.s./L)		Overall fish acute geometric mean LC_{50} (µg a.s./L)
CA8.2.1./10	[redacted], 2002	*Oncorhynchus mykiss*	96 hours (flow-through)	53.1*	Acute geometric mean for *Oncorhynchus mykiss* LC_{50} = 77 µg a.s./L	108
CA8.2.1/11	[redacted] 1991a	*Oncorhynchus mykiss*	96 hours (flow-through)	94		
CA8.2.1/14	[redacted] 2019a	*Oncorhynchus mykiss*	96 hours (flow-through)	90		
CA8.2.1/12	[redacted] 1991b	*Lepomis macrochirus*	96 hours (flow-through)	142		
CA8.2.1/13	[redacted], 2012a	*Cyprinodon variegatus*	96 hours (flow-through)	115		

** Tier 1 endpoint*

The **overall fish acute geometric mean LC_{50} value** for the 5 acute fish studies with 3 different fish species is calculated as **108 µg a.s./L**. In accordance with the procedures outlined in the EFSA Guidance Document for aquatic organisms, an **assessment factor of 100** will be applied to this geomean endpoint in determining the acute RAC value for fish, resulting in **higher tier geomean RAC of 1.08 µg MITC/L**. This RAC value will be considered in the higher tier acute risk assessment for fish.

For **aquatic invertebrates**, additional acute toxicity studies representing a range of species including crustaceans, insects, annelids, molluscs and platyhelminths are performed and included in a **species sensitive distribution**. The broad taxonomic diversity of these test species represents the use of metam as a soil sterilant.

For some taxa, more than one acute study is available. In these cases, a geometric mean acute toxicity endpoint is calculated to represent the taxon. The toxicity data of all invertebrate species are entered into a species sensitivity distribution (SSD) to reflect the range of responses to the exposure of MITC across various aquatic invertebrate species. These calculations were performed in accordance with the EFSA Guidance Document for aquatic organisms (2013). The invertebrate acute toxicity data are summarised below.

Table 2.9.9.3-6: Higher Tier: Summary of invertebrate acute toxicity studies for the active metabolite MITC and derivation of geometric mean endpoints for taxa with more than one study/endpoint available

Reference Author	Test species	Taxonomic grouping	Exposure duration (hours)	EC_{50} (µg a.s./L)	Geometric mean species EC_{50} (µg a.s./L)
CA8.2.4.2/06 [redacted], 2014a	*Hyalella azteca*	Amphipod crustacean	48 h (static)	3.8	-
CA8.2.4.2/08 [redacted]., 2011	*Americamysis bahia*	Mysid crustacean	96 h (flow-through)	55	-
CA8.2.4.1/04 [redacted], 2002	*Daphnia magna*	Cladoceran crustacean	48 h (semi-static)	76*	97
CA8.2.4.1/06 [redacted] 2019a			48 h (flow-through)	124	
CA8.2.4.2/10 [redacted], 2015	*Asellus aquaticus*	Isopod crustacean	96 h (semi-static)	110	-
CA8.2.4.2/04 [redacted], 2014c	*Chironomus riparius*	Dipteran insect	48 h (static)	360	121.5
CA8.2.4.2/05 [redacted], 2014a			48 h (semi-static)	90.6	
CA8.2.4.2/03 [redacted], 2018a			48 h (semi-static)	55	
CA8.2.4.2/15 [redacted], 2019c	*Dugesia tigrina*	Triclad platyhelminth	96 h (semi-static)	137	-
CA8.2.4.2/07 [redacted], 2014b	*Leptocheirus plumulosus*	Amphipod crustacean	48 h (static)	160	-

CA8.2.4.2/13 [redacted]., 2019a	*Lumbriculus variegatus*	Lumbricid annelid	96 h (semi-static)	315	254
CA8.2.4.2/17 [redacted] 2014c			48 h (semi-static)	205	
CA8.2.4.2/16 [redacted], 2014b	*Crangonyx pseudogracilis*	Amphipod crustacean	48 h (semi-static)	312	-
CA8.2.4.2/14 [redacted]., 2019b	*Potamopyrgus antipodarum*	Gastropod mollusc	96 h (semi-static)	319	-
CA8.2.4.2/12 [redacted], 2014b	*Brachionus calyciflorus*	Brachionid crustacean	24 h (static)	> 1200	-
CA8.2.4.2/11 [redacted]., 2014a	*Thamnocephalus platyurus*	Thamnocepahlid crustacean	24 h (static)	2000	-

** Tier 1 endpoint*

The final data entered into the SSD are summarised below.

Table 2.9.9.3-7: Higher Tier: Summary of invertebrate acute toxicity data for the metabolite MITC entered into the species sensitivity distribution

No.	Test species	Exposure duration (hours)	EC_{50} (µg a.s./L)
1	*Hyalella azteca*	48 h	3.8
2	*Americamysis bahia*	96 h	55
3	*Daphnia magna* (geomean)	48 h	97
4	*Asellus aquaticus*	96 h	110
5	*Chironomus riparius* (geomean)	48 h	121.5
6	*Dugesia tigrina*	96 h	137
7	*Leptocheirus plumulosus*	48 h	160
8	*Lumbricus variegatus* (geomean)	48 h / 96 h	254
9	*Crangonyx pseudogracilis*	48 h	312
10	*Potamopyrgus antipodarum*	96 h	319
11	*Brachionus calyciflorus*	24 h	1200
12	*Thamnocephalus platyurus*	24	2000

SSD analysis was performed with the software tool ETX 2.0 (RIVM, 2004)[37].
The median HC_5 (hazardous concentration to 5 % of the tested species that is predicted with 50 % certainty) and also the lower limit HC_5 values ($LLHC_5$; hazardous concentration to 5 % of the tested species that is predicted with 95 % certainty) were derived from the SSD curve. The **median HC_5** for aquatic invertebrates was calculated as **11.442 µg MITC/L**.
Application of an assessment factor of 6 on the median HC_5 of 11.442 µg a.s./L results in an **SSD-$RAC_{SW;AC}$ of 1.91 µg MITC/L**. This RAC will be considered in the higher tier acute risk assessment for aquatic invertebrates.

Risk assessment

[37] Van Vlaardingen PLA, Traas TP, Wintersen AM and Aldenberg T (2004). ETX 2.0, a program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. RIVM report 601501028/2004.

The risk assessment for aquatic organisms living in surface waters adjacent to treated **permanent greenhouses** is presented in Volume 3 B.9 CP Taminco. As a worst-case approach, the acute and chronic aquatic risk assessment are based on the highest PEC_{SW} value, which is the sum of drainflow and redeposition, and amounts 0.323 µg MITC/L (see Volume 3 B.9 CP Taminco).

Conclusion:
From the evaluation in Volume 3 (and the List of Endpoints) it can be concluded that the acute and chronic risk for aquatic organisms exposed to MITC from the use in permanent greenhouses can be considered acceptable. All Tier 1 (and Higher Tier) RAC values are exceeding the worst-case PEC_{SW} based on the permanent greenhouse use.

2.9.9.4 Bees

The risk assessment included in Volume 3 (PPP) sections B.9.6.1 indicated an acceptable risk to honeybees for the proposed uses of Metam Sodium 51% (both indoor and outdoor uses - Notifier: Lainco) and Metam Na 510 SL (indoor – Notifier: Taminco)

The risk assessment for effects on bees is normally based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002). However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

Table 2.9.4.4-1: Summary of bee toxicity data used in the risk assessment for metam/MITC

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Honeybees					
Honeybee (*Apis mellifera*)	MITC	48h acute toxicity test (vapour exposure for 4h)	LC_{50} (inhalation, 4h)	24 ppm (equivalent to 70770 µg MITC/m³)	CA8.3.1.1/01 [redacted]., 2016
Honeybee (*Apis mellifera*)	MITC	48h acute toxicity test (vapour exposure for 48h)	LC_{50} (inhalation, 48h)	**> 4710 µg MITC/m³**	CA8.3.1.1/02 [redacted] 2019

bold **-** *values used in the risk assessment*

Metam Sodium 51% SL (notifier: Lainco)
Due to the method of application (to bare soil, through soil injection or drip irrigation, followed by 21 days coverage with TIF), direct oral or contact exposure to bees in the field or greenhouse during application is considered to be negligible. By the time flowering weeds or crops will be present in-field, remaining MITC residues in the soil will be negligible since seeds will not germinate if significant residues are still present. Further, MITC is not systemic, and therefore oral exposure to bees at later stages of the crop growth can also be considered negligible.

In addition to the above, residues in subsequent crops (and weeds) will be negligible, as confirmed by crop residue studies (See Volume 3 (CA) Section B.7.2). Furthermore, metam is intended to be applied at a maximum of once every third year on the same field, and given the rapid breakdown of metam and MITC, no chronic exposure to adult bees or bee larvae is expected in- or off-field.

The active metabolite MITC is a highly volatile molecule. Following volatilisation from the soil, bees can potentially be exposed to vapours of MITC through inhalation. Finally, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the

treated field or greenhouse. Consequently, bees could potentially be exposed orally and through contact with these MITC deposits off-field.

The risk assessment for **off-field exposure through inhalation** was based on experimentally measured concentrations in air at the edge of the field or directly adjacent to the greenhouse. The relevant LC_{50} for honey bees following acute inhalation exposure (> 4710 µg MITC/m³) exceeds the highest measured off-field MITC concentration in air for the outdoor use through soil injection (536 µg MITC/m³) by a factor 8.8. The highest measured off-field MITC concentration for the indoor use through drip irrigation (292 µg MITC/m³) is a factor of 16.1 lower than this LC_{50} value.

As there is no standard risk assessment scheme available for inhalation exposure, there are no trigger values available to determine whether or not the risk is acceptable. However, taking into account that:

- The LC_{50} used is a greater than value. In the study by [redacted] (2016), there was a corrected mortality of only 10% at the highest concentration test (4710 µg MITC/m³).
- The measured MITC concentrations derived from the study by [redacted] (2014) are worst-case values, as no TIF was used in this study and the application rate for the indoor application was higher than the application rate requested in the GAP.

The risk to bees from exposure through inhalation of MITC is considered to be low.

The risk assessment for contact and oral **exposure through redeposition after volatilisation** (off-field) was also based on the available maximum measured concentrations of MITC in air, which were converted in g MITC/ha assuming that al the MITC present in one m³ is deposited on a 1 m² off-field area of soil (or plants). As such, for the indoor use the concentration of 292 µg MITC/m³ would result in a deposition rate of 292 µg MITC/m² which is equivalent to 2.92 g MITC/ha. For the outdoor use, the concentration of 536 µg MITC/m³ would result in a deposition rate of 536 µg MITC/m² which is equivalent to 5.36 g MITC/ha.

The worst-case off-field redeposition rate of 2.92 g MITC/ha for the indoor use represents 0.002% of the proposed in-field application rate of 306 kg metam sodium/ha (which is equivalent to 173 kg MITC/ha). The worst-case off-field redeposition rate of 5.36 g MITC/ha for the outdoor uses represents 0.006% of the proposed in-field outdoor application rate of 153 kg metam sodium/ha (equivalent to 86.6 kg MITC/ha).

No data on the oral and contact toxicity of MITC to honey bees is available, and therefore a quantitative risk assessment cannot be performed. However, the worst-case off-field redeposition was calculated to be 0.002% and 0.006% of the in-field application rate for the indoor and outdoor uses, respectively. These values were derived from a study where no TIF was used. In addition, the application rate for the indoor application was twice the application rate requested in the GAP. Given the low potential for exposure, the risk to bees from oral and contact exposure through redeposition after volatilization is considered low.

The potential risk from DMTU is considered covered by the risk assessment for MITC presented above.

Metam Na 510 SL (Notifier: Taminco)

As the requested GAP is limited to the use in permanent greenhouses, a risk assessment for bees is not required. Nevertheless, due to the method of application (to bare soil, through soil injection, followed by 6 weeks coverage with TIF), direct oral or contact exposure to (introduced) bees in the greenhouse during application is considered to be negligible. As MITC is not systemic and phytotoxic, there is also no risk for exposure of introduced pollinators to MITC in pollen and nectar of growing plants.

The active metabolite MITC is a highly volatile molecule. Following volatilisation from the soil, bees can potentially be exposed to vapours of MITC through inhalation.Finally, there is a potential for short-range redeposition of MITC after volatilisation. This could lead to contamination of the off-field area around the treated field or greenhouse. Consequently, bees could potentially be exposed orally and through contact with these MITC deposits off-field.

The risk assessment for **off-field exposure through inhalation** was based on experimentally measured concentrations in air directly adjacent to the greenhouse. The relevant LC_{50} for honey bees following acute

inhalation exposure (> 4710 µg MITC/m³) exceeds the highest measured off-field MITC concentration in air for the indoor use through drip irrigation (39.74 µg MITC/m³) by a factor of 118.

As there is no standard risk assessment scheme available for inhalation exposure, there are no trigger values available to determine whether or not the risk is acceptable. However, taking into account that:

- The LC_{50} used is a greater than value. In the study by [redacted] (2016), there was a corrected mortality of only 10% at the highest concentration test (4710 µg MITC/m³).
- There is a high margin of safety between the LC50 and the measured residues in air

The risk to bees from exposure through inhalation of MITC is considered to be low.

The risk assessment for contact and oral **exposure through redeposition after volatilization** (off-field) was based on a calculated redeposition loading of 0.00084 kg MITC/ha. This value represents 0.00097% of the in-field application rate of 153 kg metam sodium/ha (which is equivalent to 86.6 kg MITC/ha). Given the low potential for exposure, the risk to bees from oral and contact exposure through redeposition after volatilization is considered low.

2.9.9.5 Other non-target arthropods

The risk assessment included in Volume 3 (PPP) section B.9.6.2 indicated an acceptable risk to non-target arthropods. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

For both field uses and uses in permanent greenhouses and walk-in tunnels, due to the method of application to bare soil and the use of TIF, no crops or weeds will be present in-field at the time of application. Therefore, foliage-dwelling non-target arthropods will not be exposed at the time of application. As MITC is not systemic, there will be no residues in crops grown on the treated soil. Consequently, there will also be no subsequent exposure for foliage-dwelling non-target arthropods in the treated field. Therefore, the arthropods primarily at risk are those present in or on the soil.

Due to the application method of Metam Sodium 51% SL (i.e. soil injection or drip irrigation to bare soil) and Metam Na 510 SL (i.e. drip irrigation to bare soil), off-field exposure to non-target arthropods through spray drift is not relevant. However, the active metabolite MITC is a highly volatile molecule, and will be present in both the liquid and gas phase of the soil after its formation from metam. Following volatilisation from the soil, MITC can contaminate the off-field area through short-range redeposition. This route of exposure is potentially relevant for both foliage-dwelling and soil-dwelling arthropods off-field, and for both the outdoor uses and the indoor uses (in both walk-in tunnels and permanent greenhouses).

Standard laboratory studies with the indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* have not been submitted for the active substance metam, the representative formulation Metam Sodium 51% SL or the active metabolite MITC. As a general soil fumigant, metam (and MITC) is known to have insecticidal properties and adverse effects on the two indicator non-target arthropod species would be expected based on a worst-case HQ scenario with data from standard laboratory tests. In addition, the traditional test design for this type of Tier 1 studies, in which arthropods are exposed to dried residues on glass plates, is not suitable for volatile compounds such as metam/MITC applied as soil fumigants. Instead, an extended laboratory study and higher tier field studies were submitted to address the risk to non-target arthropods. These studies focussed on soil-dwelling arthropods.

Metam Sodium 51% SL (notifier: Lainco)

For the proposed use in permanent greenhouses, a risk assessment for **in-field exposure** is not necessary. For the proposed outdoor uses and the use in walk-in tunnels, a risk assessment for in-field exposure is required. This risk assessment is based on the available higher tier field study ([redacted], 2010), in which effects on soil-dwelling non-target arthropods was investigated following an application of 612 kg metam sodium/ha. Even though, in contrast to the current GAP, no TIF was used in this field study, the results are still considered useful to assess the potential for recovery of non-target arthropods. In the study by [redacted] (2010), there were clear initial effects

on arthropod populations directly after application, followed by recovery in the field within one year for the most important taxa. The application rate in the study by [redacted]. (2010) was 612 kg metam sodium/ha, which is four and two times the application rate for the currently proposed outdoor uses (153 kg metam sodium/ha) and uses in walk-in tunnels (306 kg metam sodium/ha). In addition, only one application every three years is intended on the same field. Therefore, the RMS is of the opinion that based on the available field data, it can be expected that for the currently proposed uses soil non-target arthropods will be able to recover from any initial effects within one year after application. Therefore, the risk is considered acceptable.

A quantitative risk assessment for **off-field exposure** (through redeposition) to foliage-dwelling could not be performed, due to the lack of suitable toxicity data. The available field studies for soil-dwelling arthropods are also not useful for an off-field risk assessment, as they were performed at high doses, and they take into account recovery. For the off-field area, however, the protection goal must ensure that arthropod populations are not affected at all (i.e. initial effects followed by recovery are not accepted). However, a significant exposure through redeposition after volatilization is considered unlikely, because of the coverage of the soil with TIF for at least 21 days and the low DT_{50} of MITC in soil. Worst case quantitative exposure estimates for off-field redeposition were calculated to be 2.92 g MITC/ha for the indoor use, which represents 0.002% of the proposed in-field application rate of 306 kg metam sodium/ha (which is equivalent to 173 kg MITC/ha). The worst-case off-field redeposition rate of 5.36 g MITC/ha for the outdoor uses represents 0.006% of the proposed in-field outdoor application rate of 153 kg metam sodium/ha (equivalent to 86.6 kg MITC/ha). These values are very low, and are likely an overestimation of the actual exposure, as they were derived from a study where no TIF was used after application (soil was only compressed with a roller). Overall, considering the very low expected exposure through redeposition, the off-field risk to non-target arthropods is considered acceptable even though suitable toxicity or field data is not available to perform a quantitative risk assessment.

Metam Na 510 SL (notifier: Taminco)
The GAP of Metam Na 510 SL is limited to uses in permanent greenhouses. For such uses, a risk assessment for in-field exposure is not necessary.

A quantitative risk assessment for **off-field exposure** (through redeposition) to foliage-dwelling could not be performed, due to the lack of suitable toxicity data. The available field studies for soil-dwelling arthropods are also not useful for an off-field risk assessment, as they were performed at high doses, and they take into account recovery. For the off-field area, however, the protection goal must ensure that arthropod populations are not affected at all (i.e. initial effects followed by recovery are not accepted). However, the estimated redepositing loading following the proposed use of Metam Na 510 SL is equivalent to 0.00084 kg MITC/ha. This corresponds to 0.0009% of the proposed in-field application rate of 153 kg metam sodium/ha (which is equivalent to 86.6 kg MITC/ha). Overall, considering the very low expected exposure through redeposition, the off-field risk to non-target arthropods is considered acceptable even though suitable toxicity or field data is not available to perform a quantitative risk assessment.

2.9.9.6 Earthworms

The risk assessment included in Volume 3 (PPP) section B.9.8.1 indicated an acceptable risk to earthworms.
The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

In addition to the standard risk assessment for in-field exposure, the potential risk for off-field exposure is also considered. The active metabolite MITC is a highly volatile molecule Following volatilisation from the soil, MITC can contaminate the off-field area through short-range redeposition. This route of exposure is potentially relevant for both the outdoor uses and the indoor uses (in both walk-in tunnels and permanent greenhouses).

Standard laboratory studies with the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL, or the active metabolite MITC with earthworms have not been submitted. As a general soil fumigant, adverse effects of metam (and MITC) on earthworms would be expected based on a

worst-case TER scenario with data from standard laboratory tests. Instead, higher tier field studies were submitted to address the risk to earthworms.

Metam Sodium 51% SL (notifier: Lainco)

For the proposed use in permanent greenhouses, a risk assessment for **in-field exposure** is not necessary. For the proposed outdoor uses and the use in walk-in tunnels, a risk assessment for in-field exposure is required. This risk assessment is based on the available higher tier field studies.

Two field studies on the effect of metam sodium on earthworm populations are available to the notifier (Lainco). A first study by [redacted] (2002) was conducted in Germany and tested effects of a single application of either 152.1 and 608.4 kg a.s./ha. The lowest of these two doses covers the proposed application rate for the outdoor uses (153 kg metam sodium/ha). The second study by [redacted]. (2012) was performed in Northern France, and tested effects of a single application of 196.7 and 286.1 kg a.s./ha. The highest of these two doses is considered to cover the proposed application rate for the use in walk-in tunnels (306 kg metam sodium/ha). Even though, in contrast to the current GAP, no TIF was used in these field studies, the results are still considered useful to assess the potential for recovery of earthworms following applications according to the current GAP.

Both studies included an untreated control (no test item application, no soil cultivation) and an 'agricultural control' (same soil cultivation as in the treatments, but application of water only). As also agreed during in the original DAR (2010) and during the Peer Review of the initial annex I inclusion of metam, the effects of metam sodium and its metabolite MITC should be assessed in comparison to the agricultural control rather than the untreated control. This will filter out any effects on earthworms due to soil cultivation, rather than by exposure to the test item.

Compared to the agricultural control, there were clear effects of the metam sodium treatment on earthworm abundance and biomass directly after application. However, full recovery to control levels occurred within 12 months after application. Therefore, the risk from the proposed uses of Metam Sodium 51% SL can be considered acceptable.

Estimated off-field redeposition rates were calculated to be 2.92 g MITC/ha for the indoor use, which represents 0.002% of the proposed in-field application rate of 306 kg metam sodium/ha (which is equivalent to 173 kg MITC/ha). The worst-case off-field redeposition rate of 5.36 g MITC/ha for the outdoor uses represents 0.006% of the proposed in-field outdoor application rate of 153 kg metam sodium/ha (equivalent to 86.6 kg MITC/ha). Given that the **off-field exposure** is much lower compared to the exposure in-field, the risk to soil meso- and macrofauna in the off-field area is considered covered by the risk assessment for in-field.

Metam Na 510 SL (notifier: Taminco)

The GAP of Metam Na 510 SL is limited to uses in permanent greenhouses. For such uses, a risk assessment for in-field exposure is not necessary.

The risk assessment for **off-field exposure** through redeposition is performed based on the available higher tier field studies. Two field studies on the effect of metam sodium on earthworm populations are available to the notifier (Taminco). A first study by [redacted] (2002) was conducted in Germany and tested effects of a single application of either 152.1 and 608.4 kg a.s./ha. A second study by [redacted] (2015) was performed in East-Central France and tested effects of a single application of 292.1 kg a.s./ha, 407.5 kg a.s./ha and 562.7 kg a.s./ha.

The available field studies indicate that, following initial effects on earthworm populations after application of metam sodium, recovery will occur within 12 months compared to the agricultural control. Furthermore, the magnitude of the initial effect and the duration of the time needed for recovery increases with increasing application rate of metam sodium.

The estimated redepositing loading following the proposed use of Metam Na 510 SL is equivalent to 0.00084 kg MITC/ha. This corresponds to 0.0009% of the proposed in-field application rate of 153 kg metam sodium/ha (which is equivalent to 86.6 kg MITC/ha). As the application rates tested in the available field studies largely exceed the estimated redepositing loading following the proposed use of Metam Na 510 SL, the off-field risk to earthworms can be considered acceptable.

2.9.9.7 Other soil macro-organisms

The risk assessment included in Volume 3 (PPP) section B.9.8.2 indicated an acceptable risk to other soil meso- and macrofauna. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). However, this guidance document does not cover special substances such as soil fumigants like metam. A specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

In addition to the standard risk assessment for in-field exposure, the potential risk for off-field exposure is also considered. The active metabolite MITC is a highly volatile molecule Following volatilisation from the soil, MITC can contaminate the off-field area through short-range redeposition. This route of exposure is potentially relevant for both the outdoor uses and the indoor uses (in both walk-in tunnels and permanent greenhouses).

Standard laboratory studies with the indicator species *Hypoaspis aculeifer* and *Folsomia candida* have not been submitted for the active substance metam, the representative formulations Metam Sodium 51% SL and Metam Na 510 SL, or the active metabolite MITC. As a general soil fumigant, adverse effects of metam and/or MITC on the two soil indicator species would be expected based on a worst-case TER scenario with data from standard laboratory tests. Instead, higher tier field studies were submitted to address the risk to non-target soil meso- and macrofauna.

Metam Sodium 51% SL (notifier: Lainco)

For the proposed use in permanent greenhouses, a risk assessment for **in-field exposure** is not necessary. For the proposed outdoor uses and the use in walk-in tunnels, a risk assessment for in-field exposure is required. This risk assessment is based on the available higher tier field study ([redacted], 2010), in which effects on soil meso- and macrofauna was investigated following an application of 612 kg metam sodium/ha. Even though, in contrast to the current GAP, no TIF was used in this field study, the results are still considered useful to assess the potential for recovery of non-target arthropods.

In the study by [redacted] (2010), there were clear initial effects on soil meso- and macrofauna populations directly after application, followed by recovery in the field within one year for the most important taxa. The application rate in the study by [redacted] (2010) was 612 kg metam sodium/ha, which is four and two times the application rate for the currently proposed outdoor uses (153 kg metam sodium/ha) and uses in walk-in tunnels (306 kg metam sodium/ha). In addition, only one application every three years is intended on the same field. Therefore, the RMS is of the opinion that based on the available field data, it can be expected that for the currently proposed uses soil non-target soil meso- and macrofauna will be able to recover from any initial effects within one year after application. Therefore, the risk is considered acceptable.

Estimated off-field redeposition rates were calculated to be 2.92 g MITC/ha for the indoor use, which represents 0.002% of the proposed in-field application rate of 306 kg metam sodium/ha (which is equivalent to 173 kg MITC/ha). The worst-case off-field redeposition rate of 5.36 g MITC/ha for the outdoor uses represents 0.006% of the proposed in-field outdoor application rate of 153 kg metam sodium/ha (equivalent to 86.6 kg MITC/ha). Given that the **off-field exposure** is much lower compared to the exposure in-field, the risk to soil meso- and macrofauna in the off-field area is considered covered by the risk assessment for in-field.

Metam Na 510 SL (notifier: Taminco)

The GAP of Metam Na 510 SL is limited to uses in permanent greenhouses. For such uses, a risk assessment for in-field exposure is not necessary.

The risk assessment for **off-field exposure** through redeposition is performed based on the available higher tier field study by [redacted] (2010), in which effects on soil meso- and macrofauna was investigated following an application of 612 kg metam sodium/ha. This study indicates that, following initial effects on soil meso- and macrofauna populations after application of metam sodium, recovery will occur within 12 months.

The estimated redepositing loading following the proposed use of Metam Na 510 SL is equivalent to 0.00084 kg MITC/ha. This corresponds to 0.0009% of the proposed in-field application rate of 153 kg metam sodium/ha (which is equivalent to 86.6 kg MITC/ha). As the application rates tested in the available field studies largely exceed the estimated redepositing loading following the proposed use of Metam Na 510 SL, the off-field risk to non-target soil meso- and macrofauna can be considered acceptable.

2.9.9.8 Soil micro-organisms

The risk assessment included in Volume 3 (PPP) section B.9.10 indicated an acceptable risk to soil nitrogen transformation processes. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). However, this guidance document does not entirely cover special substances such as soil fumigants like metam. Therefore, an additional specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

In addition to the standard risk assessment for in-field exposure, the potential risk for off-field exposure is also considered. The active metabolite MITC is a highly volatile molecule Following volatilisation from the soil, MITC can contaminate the off-field area through short-range redeposition. This route of exposure is potentially relevant for both the outdoor uses and the indoor uses (in both walk-in tunnels and permanent greenhouses).

Laboratory toxicity studies on the effect of the active substance metam, the representative formulation Metam Sodium 51% SL or the active metabolite MITC on soil nitrogen transformation have not been submitted. As metam is a general soil fumigant, a significant impact on soil microbial activity is inherent to the active substance. Therefore, the notifier proposed not to submit a standard laboratory test according to OECD 216. Instead, a higher tier laboratory study, using aged soil samples collected from a treated field has been submitted to address the risk to soil nitrogen transformation.

Metam Sodium 51% SL (notifier: Lainco)

For the proposed use in permanent greenhouses, a risk assessment for **in-field exposure** is not necessary. For the proposed outdoor uses and the use in walk-in tunnels, a risk assessment for in-field exposure is required. This risk assessment is based on the available higher tier laboratory study by [redacted] (2002). In this study, the effect on soil nitrogen and carbon transformation was investigated using soil samples from the earthworm field study by [redacted] (2002). The test plots from which these soil samples were collected were treated with either 152.1 or 608.4 kg metam sodium/ha. Compared to the untreated control, the soil nitrogen turnover was affected by the metam sodium application up to day 56 after application. At 102 and 184 day after application, the nitrogen turnover in the treated plots was still somewhat different from the control, but these differences were no longer statistically significant. Even though high variations were observed in between replicates from the untreated control and the agricultural control, the study was considered acceptable in the initial DAR (2010). Overall, it was concluded in the original DAR (2010), and confirmed in the EFSA Conclusion (EFSA Journal 2011;9(9):2334) that based on this study, the effects of metam sodium and its metabolite MITC on nitrogen and carbon transformation were transient and the processes were not drastically impaired up to an application rate of 612 kg a.s./ha.

The application rate for proposed uses of Metam Sodium 51% SL is limited to 153 kg a.s./ha for the outdoor uses and 306 kg a.s./ha for the indoor uses. These application rates are both covered by the rates tested in the study by [redacted] (2002). Therefore, the risk to soil micro-organisms following these proposed uses can be considered acceptable.

Estimated off-field redeposition rates were calculated to be 2.92 g MITC/ha for the indoor use, which represents 0.002% of the proposed in-field application rate of 306 kg metam sodium/ha (which is equivalent to 173 kg MITC/ha). The worst-case off-field redeposition rate of 5.36 g MITC/ha for the outdoor uses represents 0.006% of the proposed in-field outdoor application rate of 153 kg metam sodium/ha (equivalent to 86.6 kg MITC/ha). Given that the **off-field exposure** is much lower compared to the exposure in-field, the risk to soil meso- and macrofauna in the off-field area is considered covered by the risk assessment for in-field.

Metam Na 510 SL (notifier: Taminco)
The GAP of Metam Na 510 SL is limited to uses in permanent greenhouses. For such uses, a risk assessment for in-field exposure is not necessary.

The risk assessment for **off-field exposure** through redeposition is performed based on the available higher tier laboratory study by [redacted] (2002). As explained above, the effects of metam sodium and its metabolite MITC on nitrogen and carbon transformation in this study were transient and the processes were not drastically impaired up to an application rate of 612 kg a.s./ha.

The PEC_{soil} for redeposition off-field for the proposed indoor use of Metam Na 510 SL was compared with the PEC_{soil} for the lowest application rate of 152.1 kg metam sodium/ha in the study by [redacted] (2002) (see Table 2.9.9.8-1), which indicated that the exposure in the field study largely exceeded the expected exposure through redeposition. Taking into account the results from [redacted] (2002), and the very low level of exposure following redeposition off-field, the risk to soil micro-organisms is considered acceptable.

Table 2.9.9.8-1: Predicted environmental concentrations of MITC in soil for the proposed GAP use of metam and estimated for the lowest application rate in the earthworm field study by ([redacted], 2002; CP 10.4.1.2/01)

Application Scenario	Application rate (g MITC/ha)	Incorporation depth (cm)	Fraction intercepted by crop	Dry bulk density (g cm^3)	PEC_{soil} (mg MITC/kg)
Metam GAP	0.84	5	0	1.5	0.00112
Field study ([redacted], 2002)	86300	25	0	1.5	23.01

2.9.9.9 Non-target plants

The risk assessment included in Volume 3 (PPP) section B.9.12 indicated an acceptable risk to non-target plants. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). However, this guidance document does not entirely cover special substances such as soil fumigants like metam. Therefore, an additional specific risk assessment for the exposure routes that are considered relevant for the proposed uses of metam sodium was performed.

The potential risk from DMTU is considered covered by the risk assessment for MITC.

For the proposed methods of soil application, spray drift is not a relevant route of exposure. However, the active metabolite MITC is ha highly volatile molecule, and will be present in both the liquid and gas phase of the soil after its formation from metam. Following volatilisation from the soil, off-field exposure to non-target plants can occur through MITC vapours. In addition, there is a potential for short-range redeposition of MITC after volatilisation, which could lead to contamination of the off-field area around the treated field or greenhouse. Consequently, non-target plants could also be exposed to these MITC deposits off-field.

Metam Sodium 51% SL (notifier: Lainco)
The risk assessment for **vapour exposure** was based on experimentally measured concentrations in air at the edge of the field or directly adjacent to the greenhouse. For the indoor use, the calculated TER of 5.14 (see Table 2.9.9.9-1) exceeds the trigger of 5, indicating an acceptable risk.

For the outdoor use, the TER value calculated for the worst case maximum measured air concentration, directly at the edge of the field, was 2.80, which indicates a potential risk. However, it should be noted that the vegetative vigour study by [redacted] (2013) represented a very worst-case exposure scenario, in which plants were subjected to 4 consecutive 12.5-hour exposures periods to MITC vapour. Constant off-field exposure to non-target terrestrial plants over a 50 hour (i.e. 2 day) period to MITC in air is highly unlikely following the proposed use. In addition, the worst-case measured air concentrations are derived from a study where no TIF was used. According to the GAP, the soil should be covered with TIF for a period of 21 days, which will result in a significantly lower volatilization. Refining the exposure estimate by calculating the 75^{th} percentile of all 24 measurements at 0 m and 5 m from the field results in a $PEC_{off\text{-}field}$ of 281 µg MITC/m^3. The TER based on this still conservative exposure value is 5.34, which indicates an acceptable risk.

Table 2.9.9.9-1: TER values based on worst-case values for exposure to MITC vapours

Crop	EC_{50} (µg MITC/m³)	$PEC_{off\text{-}field}$ (µg MITC/m³)	TER	Trigger
Potato / Carrot / Onion (outdoor)	1502	536 – worst-case maximum (0 m) [1]	**2.80**	5
		281 – 75th percentile (0-5 m) [2]	5.34	
Pepper (indoor)		292 – worst-case maximum (0 m) [1]	5.14	

[1]values measured at the edge of the treated area for the outdoor use, and directly adjacent to the greenhouse plastic for the indoor use,; [2]75th percentile air concentration based on measured data for 24 bystanders at 0 and 5m from the field, TER values in bold are below the trigger and indicate a potential risk

The risk assessment for **exposure through redeposition** was also based on the available maximum measured concentrations of MITC in air, which were converted in g MITC/ha assuming that al the MITC present in one m³ is deposited on a 1 m² off-field area of soil (or plants). For both the indoor and outdoor uses, the calculated TER values exceed the trigger of 5, indicating an acceptable risk.

Table 2.9.9.9-2: TER values based on worst-case values for exposure to MITC through redeposition

Crop	EC_{50} (mg MITC/kg soil dw)	$PEC_{off\text{-}field}$ (mg MITC/kg soil dw)	TER	Trigger
Potato / Carrot / Onion (outdoor)	5.76	0.00715	805.6	5
Pepper (indoor)		0.00389	1480.7	

Metam Na 510 SL (notifier: Taminco)

The risk assessment for **vapour exposure** was based on experimentally measured concentrations in air directly adjacent to the greenhouse. For the proposed indoor use, the calculated TER exceeds the trigger of 5, indicating an acceptable risk.

Table 2.9.9.9-3: TER values for exposure to MITC vapours

Crop	EC_{50} (µg MITC/m³)	$PEC_{off\text{-}field}$ (µg MITC/m³)	TER	Trigger
Lettuce/ Ornamentals/ Baby leaf	1502	39.74	37.8	5

The risk assessment for contact and oral **exposure through redeposition after volatilization** (off-field) was based on a calculated redeposition loading. For the proposed indoor use, the calculated TER exceeds the trigger of 5, indicating an acceptable risk.

Table 2.9.9.9-4: TER values for exposure to MITC through redeposition

Crop	EC_{50} (mg MITC/kg soil dw)	$PEC_{off\text{-}field}$ (mg MITC/kg soil dw)	TER	Trigger
Lettuce/ Ornamentals/ Baby leaf	5.76	0.00112	5142.9	5

2.9.9.10 Biological methods for sewage treatment

The risk assessment included in Volume 3 (PPP) Section B.9.14.1 indicated an acceptable risk for biological methods for sewage treatment.

the EC_{50} for MITC produced in the activated sludge test was 0.990 mg/L (based on initial measured concentrations) or 0.311 mg/L (based on mean measured concentrations). These EC_{50} values exceed the calculated overall

maximum PEC_{SW} of 0.323 µg MITC/L for the proposed indoor uses Metam Na 510 SL. As dilution prior to reaching sewage treatment works would also be expected to reduce the exposure further, the risk to sewage treatment facilities is considered limited.

2.10 ENDOCRINE DISRUPTING PROPERTIES

The notifier submitted a position paper, in which an assessment for the potential for endocrine disruption in non-target organisms, in line with the ECHA/EFSA Guidance Document (2018), was performed. This position paper is included below, followed by the evaluation and conclusion by the RMS. Together with the position paper, an Excel file according to the template published as Appendix E to the ECHA/EFSA Guidance Document (updated version of 20 February 2019) was submitted, both for metam and MITC. These Excel files are submitted as separate files.

Report author:	[redacted]
Report year:	2019
Report title:	Assessment of endocrine disrupting properties of metam and MITC – Appendix I
Report No.:	108374-CA5-3
Document No.:	-
Guidelines followed in study:	Not applicable
Deviations from current test guideline:	-
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Not applicable
Acceptability/Reliability:	This study is acceptable
Study owner:	Taminco BV, Lainco S.A.

Note: the initial version of this position paper was dated 8 December 2019. An updated version, dated 20 October 2020, has been submitted by the notifier during the course of the evaluation process. Compared to the initial version, the updated version only contained some additional information on the progress of the initiated additional studies.

Note: in order to facilitate the cross-reference to the various studies in Vol.3, B.6 and B.9, following **table 2. 10a** (Metam studies) and **table 2. 10b** (MITC studies), were drafted with the study identifiers (ID numbers) and all study characteristics.
However, it is of note that regarding the ToxCast21 (EDSP *in-vitro* assays) RMS evaluated more studies than those highlighted by the notifier. These assays are not considered overly positive for the EATS modalities, but they were added in this evaluation for reasons of transparency.

Table 2. 10a Metam studies

Study ID	Study	Study reference	Guideline	Reference
1	Subacute 8d (RF) oral (drinking water) in C57BL mouse	[redacted]	n.a.	[redacted], 1990
2	Chronic toxicity oral (capsule) 1-yr dog	[redacted]	EPA 83-1; ~ OECD 452	[redacted], 1994
3	Repeated dose 90-day oral (capsule) toxicity study dog	[redacted]	EPA 82-1; ~ OECD 409	[redacted], 1992
4	Repeated dose 90-day oral (drinking water) toxicity study in rat	[redacted]	EPA 82-1; ~ OECD 408	[redacted], 1991
5	Repeated dose 90-day oral (capsule) toxicity study in dog	[redacted]	none	[redacted] 1993
6	Repeated dose 90-day oral (drinking water) toxicity study in C57BL mouse	[redacted]	~ OECD 408	[redacted] 1991
7	Subacute 21d oral (drinking water) in rat	[redacted]	~ OECD 407	[redacted], 1991
8	Subacute 21d inhalation toxicity in SD rat	[redacted]	~ OECD 413	[redacted] 1979
9	Subchronic 90d inhalation toxicity in SD rat	[redacted]	~ OECD 412	[redacted], 1983
10	Repeated dose 21d dermal toxicity in WR rabbit	[redacted]	~ OECD 410	[redacted], 1979
11	Carcinogenicity in Wistar rat	[redacted]	EPA 83-5; ~ OECD 453	[redacted], 1994
12	Carcinogenicity oral (drinking water) in C57 BL mouse	[redacted]	EPA 83-2; ~ OECD 451	[redacted], 1994
13	Prenatal developmental toxicity RF-study (oral) in Wistar rat	[redacted]	~ OECD 414	[redacted] 1993a
14	Prenatal developmental toxicity study (oral) in Wistar rat	[redacted]	OECD 414	[redacted], 1993b
15	Prenatal developmental toxicity study (oral) in Wistar rat	[redacted]	OECD 414	[redacted], 1987a
16	Prenatal developmental toxicity study (oral) in Himalayan rabbit	[redacted]	OECD 414	[redacted], 1987b
17	Prenatal developmental toxicity study (oral) in NZW rabbit	[redacted]	OECD 414	[redacted], 1993c
18	Multigenerational reproductive toxicity study (oral) in Wistar rat	[redacted]	OECD 416 (1983)	[redacted], 1993
19	InVitroToxCast Estrogen ToxCast ER prediction model			
20	InVitroToxCast Androgen ToxCast AR prediction model			
21	InVitroToxCast Steroidogenesis TOX21_Aromatase_Inhibition Human breast cell line, MCF-7			
22	InVitroToxCast Steroidogenesis TOX21_Aromatase_Inhibition viability Human breast cell line, MCF-7			
23	InVitroToxCast Thyroid ATG_THRa1_TRANS_up Human liver cell line, HepG2			
24	InVitroThyroid Other TR in vitro assay Rat, NVS_GPCR_rTRH cell-free rat forebrain membranes			
25	InVitroThyroid Other TR in vitro assay Rat, NCCT_TPO_AUR_dn thyroid gland tissue based, cell-free assay			
26	InVitroThyroid Other TR in vitro assay, TOX21_TSHR_HTRF_Agonist_ratio Human kidney cell line, HEK293T			
27	InVitroThyroid Other TR in vitro assay, TOX21_TSHR_HTRF_Antagonist_ratio Human kidney cell line, HEK293T			

Study ID	Study	Study reference	Guideline	Reference
28	InVitroToxCast Thyroid Tox21_TR_LUC_GH3_Agonist Rat pituitary gland cell line, GH3			
29	InVitroToxCast Thyroid Tox21_TR_LUC_GH3_Antagonist Rat pituitary gland cell line, GH3			
30	InVitroThyroid Other TR in vitro line, TOX21_TR_LUC_GH3_Antagonist_viability Rat pituitary gland cell line, GH3			
31	InVitroThyroid Other TR in vitro assay ATG_THRa1_TRANS_dn Human liver cell line, HepG2			
32	InVitroThyroid Other TR in vitro assay NCCT_HEK293T_CellTiterGLO* Human kidney cell line, HEK293T			
33	InVitroThyroid Other TR NCCT_QuantiLum_inhib_2_dn in vitro assay gene-proteins from E. coli, cell-free assay			
34	InVitroThyroid Other TR in vitro assay TOX21_TSHR_wt_ratio Human kidney cell line, HEK293T			
35	Subchronic 90d oral (drinking water) neurotoxicity in SD rat	[redacted]	~ OECD 424	[redacted] 1994
36	Prenatal developmental toxicity study (RF1) in NZW rabbit	[redacted]	n.a.	[redacted], 1993a
37	Prenatal developmental toxicity study (RF2) in NZW rabbit	[redacted]	n.a.	[redacted], 1993b

*RMS : found as CCTE_Simmons_CellTiterGLO_HEK293T

Table 2. 10b MITC studies

Study ID	Study	Study reference	Guideline	Reference
1	Repeated dose 90-day oral toxicity study dog	[redacted]	OECD 409	[redacted], 1986
2	Subacute inhalation toxicity, wistar rat	[redacted]	OECD 412 (1981)	[redacted], 1987
3	Subacute inhalation toxicity, RF, CD rat	[redacted]	n.a.	[redacted], 2011
4	Subacute inhalation toxicity, B6C3F1, CD-1 mouse	[redacted]	n.a.	[redacted], 2013
5	Prenatal developmental toxicity study, oral (gavage) Wistar rat	[redacted]	OECD 414	[redacted], 1987
6	Prenatal developmental toxicity study, oral (gavage), CH rabbit	[redacted]	OECD 414	[redacted], 1986
7	Prenatal developmental toxicity main study, inhalation, NZW rabbit	[redacted]	OECD 414	[redacted] 2012c
8	Prenatal developmental toxicity RF2 study, inhalation, NZW rabbit	[redacted]	n.a.	[redacted], 2012b
9	Prenatal developmental toxicity RF1 study, inhalation, NZW rabbit	[redacted]	n.a.	[redacted], 2012a
10a	Reproduction/developmental toxicity screening test, inhalation, CD rat, F_0 adult	[redacted]	OECD 421	[redacted] 2013a
10b	Reproduction/developmental toxicity screening test, inhalation, CD rat, F_1 offspring	[redacted]	OECD 421	[redacted], 2013a
11	Two-generation reproduction toxicity test oral CD rat	[redacted]	OECD 416 (1983)	[redacted], 1987
12	Two-generation reproduction toxicity test, inhalation, CD rat	[redacted]	OECD 416 (2001)	[redacted], 2014
13	Prenatal developmental toxicity study, RF, inhalation, CD rat	[redacted]	n.a.	[redacted], 2013b
14	Prenatal developmental toxicity main study, inhalation, CD rat	[redacted]	OECD 414	[redacted], 2012d
15	Carcinogenicity,18 months inhalation, CD mouse	[redacted]	OECD 451	[redacted], 2015b
16	Carcinogenicity, 24 months, inhalation, CD rat	[redacted]	OECD 453	[redacted], 2015a
17	Carcinogenicity, 106 weeks, oral (drinking water), ICR mouse	[redacted]	~ OECD 453	[redacted] 1980
18	Carcinogenicity, oral (drinking water), CD rat	[redacted]	~ OECD 453	[redacted], 1984
19	Subchronic, 90d, inhalation, CD rat	[redacted]	U.S. EPA OPPTS 870.3465 ~ OECD 413	[redacted], 2012
20	Subchronic, 90d, inhalation, CD mouse	[redacted]	U.S. EPA OPPTS 870.3465 ~ OECD 413	[redacted], 2013
21	ToxCast ER prediction model			
22	ToxCast AR prediction model			
23	NVS_ADME_hCYP19A1 Human, cell-free			
24	TOX21_Aromatase_Inhibition Human breast cell line, MCF-7			
25	TOX21_Aromatase_Inhibition_viability Human breast cell line, MCF-7			
26	ATG_THRa1_TRANS_up Human liver cell line, HepG2			
27	NVS_NR_hTRa_Antagonist			

Study ID	Study	Study reference	Guideline	Reference
	Human, cell-free			
28	Other TR in vitro assay TOX21_TSHR_Agonist_ratio Human kidney cell line, HEK293T			
29	Other TR in vitro assay TOX21_TSHR_Antagonist_ratio Human kidney cell line, HEK293T			
30	Tox21_TR_LUC_GH3_Agonist Rat pituitary gland cell line, GH3			
31	Tox21_TR_LUC_GH3_Antagonist Rat pituitary gland cell line, GH3			
32	Other TR in vitro assay Tox21_TR_LUC_GH3_Antagonist_viability Rat pituitary gland cell line, GH3			
33	Other TR in vitro assay ATG_THRa1_TRANS_dn Human liver cell line, HepG2			
34	Other TR in vitro assay TOX21_TSHR_wt_ratio Human kidney cell line, HEK293T			
35	Subacute 28d, inhalation, immunotoxicity	[redacted]	OECD 412	[redacted] 2011b
36a	Fish *Oncorhynchus mykiss*	[redacted]	OECD 204	[redacted]
37a	Fish early life stage toxicity test *Pimephales promelas*	[redacted]	OECD 2010, US EPA OPPTS 850.1400	[redacted]
37b	Fish early life stage toxicity test *Pimephales promelas*	[redacted]	OECD 2010, US EPA OPPTS 850.1400	[redacted]
37c	Fish early life stage toxicity test *Pimephales promelas*	[redacted]	OECD 2010, US EPA OPPTS 850.1400	[redacted]
37d	Fish early life stage toxicity test *Pimephales promelas*	[redacted]	OECD 2010, US EPA OPPTS 850.1400	[redacted]
38a	*Daphnia magna* test	99/0547/51/2	OECD 202	[redacted]
38b	*Daphnia magna* test	99/0547/51/2	OECD 202	[redacted]

1. Gather all relevant information

1.1. Literature search

A comprehensive literature search for toxicology and ecotoxicology was performed. For details on the literature search, please refer to the related Literature Review Report (LRR) in the dossier (summarised in Vol.3 B.6.10.1 and B.9.11). The search was conducted in accordance with provisions of the new ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (2018), Annex F.

Briefly, as first step a single-search was conducted. During an expert evaluation, 135 titles for toxicology and 142 titles for ecotoxicology were rated as relevant/potentially relevant for general (eco)toxicity (not exclusively with regard to potential endocrine disrupting properties) and the respective abstracts downloaded. From this selection, 5 abstracts for toxicology and 9 abstracts for ecotoxicology were assessed as relevant/potentially relevant with regard to potential endocrine disrupting properties and full-text publications ordered. These publications were evaluated in detail with regard to their reliability and relevance.

For toxicology,
-there were a number of articles from the open scientific literature which focused on the *in-vitro* assessment of EATS modalities. However, these are reports from the US-EPA on high-throughput screenings of **EATS-driven** effects, for which the outcome was directly extracted from the EDSP website, and which were and listed in Vol.3 (B.6.10.2/02 and – 03). This analysis is summarised and evaluated in B.6.8.3/03 and further integrated in the LoE in this vol.1 part.
-Two other publications pertain to **non-EATS modalities**, and highlight the effect of metam on the metabolism of hypothalamic catecholamines. The studies are summarised in B.6.8.2.3 (Goldman 1994 and 2007).
RMS thinks that in the absence of overt adverse findings on fertility and endocrine-sensitive organs in the guideline studies, mediated through EATS mode of action (apart from effects associated with systemic toxicity, caused by both metam and MITC in mammals potentially exposed via oral or inhalatory route), the intrinsic capability of metam to interfere with catecholamines is for the time being based on insufficient *in-vivo* evidence to consider the a.s. and its main metabolite endocrine disruptors.
The potential effect on catecholamines by dithiocarbamates in general and metam in particular is certainly not entirely resolved and thus subject to further future investigations.

For ecotoxicology, no publication was rated as relevant and reliable to be included into the subsequent assessment for potential endocrine disrupting properties. The rationale for relevance and reliability is included in the literature evaluation documentation (please refer to the related LRR of the different sections).

Besides, a non-STN database screening was conducted for metam. The results are compiled in the overview below (Table 2.10-1), metam is included in the following lists:

- EU priority list (ED cat 1 - at least one study providing evidence of endocrine disruption in an intact organism, not a formal weight of evidence approach);
- EU Impact assessment screening study (unclassified).

With regard to the EU priority list, it has to be noted that this database is not recent and actually relevant, but included for completeness sake. With regard to the EU Impact assessment screening report, the following should be noted:
The results of the screening do not constitute evaluations of individual substances to be carried out under the respective chemical legislations [in particular, Regulation (EC) No 1107/2009 on plant protection products, Regulation (EU) No 528/2012 on biocidal products, Regulation (EC) No 1907/2006 REACH, Regulation (EC) No 1223/2009 on cosmetic products and the Water Framework Directive (EC) No 2000/60] and in no way prejudge future decisions on active substances to be taken pursuant to these pieces of the EU legislation.
It would thus be erroneous to consider that the substances listed in the results of this study (SANTE/2015/E3/SI2.706218) are considered as endocrine disruptors within the meaning of the EU legislation.

Table B.2.10-1: Non-STN database screening results for metam

		RMS remark
Substance	Metam	
CAS	137-42-8	

Candidate list of SVHCs	N	
CoRAP list	N	
ECHA ED assessment list	N	
Priority list EU	Y: ED cat 1	
European Commission impact assessment	Y: Unclassified	
EDSP 21 lists	N	
C&L Carc/ Repro/ STOT RE	N	Y*
PACT	N	

*Y: yes, N: no; * proposal during evaluation*

Note RMS: a table was compiled on the basis of reported «active» or «borderline / weak / non-concentration related response /inactive » outcomes in the EDSP database of either **metam** or its main metabolite **MITC**, taking into account a complete rehearsal of the data found for these a.s. on the following webpages:
Metam[1]: https://comptox.epa.gov/dashboard/chemical/concentration-response-data/DTXSID2029167
MITC[2]: https://comptox.epa.gov/dashboard/chemical/concentration-response-data/DTXSID2027204
More details are found in tables B.6.8.3/03-1 and -2: summary of ED *in-vitro* assay response of Metam and MITC (selected assays) obtained in US EPA EDSP screening, of vol.3 B.6.

1.2. *In silico* screening for potential endocrine disrupting properties
Following the recommendations given in Annex D of the ED Guidance, an *in silico* screening for potential endocrine disrupting properties and endocrine activity of metam and MITC was performed. For details please refer to the respective report (B.6.8.3/02 ([redacted] 2019) in Vol.3 B.6.8.3.

Briefly, (Q)SAR predictions were generated using selected publicly available and commercial models. Five QSAR tools were applied for predictions of potential endocrine activity of metam and MITC: OECD QSAR Toolbox, Vega, COSMOS, Danish QSAR database and ToxCast COMPARA/CERAPP consensus models. The following EATS-related parameters were addressed estrogen receptor (ER), androgen receptor (AR), thyroid receptor (TR), glycocorticoid receptor (GR); and parameters linked to other mode of actions (liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR), retinoid acid receptor (RAR), retinoid X receptor (RXR), aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), CYP3A4, Farnesoid X receptor (FXR), Progesterone receptor (PR), vitamin D receptor (VDR).

Experimental results for both parent substance metam and its metabolite MITC were present in some training sets for in silico models. The general outcome of the in silico screening for metam and MITC is shortly summarised in Table 2.10-2.

Table 2.10-2: Summary of (Q)SAR outcome of *in silico* screening of metam and MITC

Modality	Evaluation	Remarks
Oestrogen	Low potential	Due to the high amount of data available on ER activity and the high quality of CERAPP Consensus predictions the assessment of ER activity of Metam and Methyl Isothiocyanate is considered reliable. The CERAPP consensus predictions coincide well with the output of the AUC model.
Androgen	Low potential	Due to the quality of COMPARA consensus predictions in combination with other models (predicting no androgenic activity), the assessment of androgenic activity based on the available models is considered reliable for Metam and Methyl Isothiocyanate.
Thyroid	Inconclusive	There are only a few models available for thyroid receptor. While the molecular docking method (COSMOS) indicates low probability of binding, two models available in the Danish QSAR database yield positive results for Metam and inconclusive results for the metabolite Methyl Isothiocyanate. Both compounds are present in the training sets of the models, and the experimental value is positive in case of Metam (as well as for two similar substances, Metam Potassium and Methylcarbamic Acid), and it is negative in case of Methyl Isothiocyanate. Without further evidence, the overall outcome of the in silico screening on thyroid activity is thus considered to be inconclusive

Steroid	Low potential	Only one model is available for steroid receptors: glucocorticoid receptor (GR). For both compounds, steroid activity is evaluated by means of the molecular docking method (COSMOS). The results indicate low probability of binding. Given the lack of other models the result should be considered with caution.

RMS: confirms that EAS-modalities come out negative in the *in-silico* predictions. Regarding the T-modality, a positive (metam) and an inconclusive outcome is found. The positive finding in metam concerns the result in the ToxCast chemicals screened using AUR-TPO assay. 1074 ToxCast chemicals were screened using a single, high concentration in the AUR-TPO assay. Activity is presented as the mean of 3 biological replicates such that vehicle control is 0% (no inhibition) and 100% indicates maximal inhibition. In this publication, the average inhibition of a well-known disruptor for TPO, namely ethylene thiourea (ETU) amounts to about 81%, while the figures are about 44% for metam-sodium and as low as 2% for the main breakdown product MITC. Expectedly, the QSAR outcome based on this training set is positive as well. Compared to metam, the metabolite ETU is an environmental degradation product, a metabolite and an impurity in ethylenebisdithiocarbamate fungicides such as mancozeb, maneb and zineb, but not of metam. Further *in-vivo* data indicate that metam is unlikely to be a primary thyroid toxicant.

Overall, *in-silico* data may be an essential dataset for substances without higher-tier studies, but as the latter are present in the metam/MITC EU dossier, the ED assessment via *in-silico* techniques are at best considered providing complementary information.

2. ED assessment for humans

The assessment follows the strategy as laid down in the new ECHA/EFSA ED Guidance. All available data (i.e. available repeated dose toxicity studies in mammals, in vitro mechanistic data, in silico information) on metam and its degradation product methyl isothiocyanate (MITC) were considered for assessment.

2.1. ED assessment for T-modality

Information summarised in Table Annex E and the resulting data matrix is considered as reliable and relevant. Toxicological studies are conducted according to GLP as well as OECD test guidelines and in vitro data was extracted from the EPA database CompTox. None of the published studies have been considered relevant/reliable and therefore, were not included into Table E and the Lines of Evidence (LoE). The rationale for relevance and reliability is included in the literature evaluation documentation (please refer to the related Literature Review Report).

2.1.1 Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated parameters	Based on the requirements of the ECHA/EFSA ED Guidance, potential T-mediated adversity has been sufficiently investigated for metam and MITC as thyroid weight and/or histopathology were addressed in repeated dose toxicity studies. T-mediated potential endocrine activity has not been sufficiently investigated according to the ECHA/EFSA ED Guidance as the following parameters were not addressed: - Thyroid hormone levels (hormone levels were not addressed in repeated dose toxicity (RDT) studies as the studies were performed according to former OECD guideline versions). However, as thyroid weights and histopathology were investigated in RDT studies and no T-mediated adversity was observed, a conclusion on potential T-mediated endocrine properties based on the available data is possible and thus, the dataset on potential T-mediated adversity is considered sufficient.

2.1.2 Lines of evidence for adverse effects and endocrine activity related to T-modality

2.1.2.1 Metam

Lines of evidence for the T-modality are included in **table 2.1.2.1-1.**
RMS also revised and expanded the *in-vitro* data extracted from the EDSP database of the US EPA.
Details of the evaluation of the EDSP *in-vitro* studies (US EPA) are in Vol.3 B.6.8.3.

Table 2.1.2.1-1.: METAM Lines of evidence of THYROID modality: *in-silico, in-vitro,* and *in-vivo* studies.

ID	Effect classifi-cation	Effect target	Species / cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence (conclusions)
n.a.	In silico prediction	QSAR prediction	n.a.	n.a.	n.a.	n.a.	**Change**	Danish QSAR DB: Thyroperoxidase (TPO) inhibition QSAR (Rat in vitro) (based on positive experimental result from training set; training set is NCCT data also provided in CompTox Database, see below)	(1) Summary conclusion on potential thyroid activity: **Inconclusive**
n.a.	In silico prediction	QSAR prediction	n.a.	n.a.	n.a.	n.a.	No effect	COSMOS: THR: no potential NR ligand found; no indication for thyroid receptor activity	
23	In vitro mechanistic	Thyroid receptor	Human liver cell line, HepG2	24h	Uptake from the medium (in vitro)		No effect		(2) Based on the negative outcome of in vitro screening assays, the endocrine activity related to T-modality is assessed as negative. Potential TPO inhibition is assessed as **inconclusive but not likely based on the chemical structure.**
24	In vitro mechanistic	Thyroid receptor	Rat, cell-free rat forebrain membranes	4h	Uptake from the medium (in vitro)		No effect		
25	In vitro mechanistic	Thyroid receptor	Rat, thyroid gland tissue based, cell-free assay	0.5h	Uptake from the medium (in vitro)	20µM	**Change**	NCCT_TPO_AUR_dn (Thyroperoxidase) Biological process target: regulation of catalytic activity Flags: < 50% efficacy; Only highest conc > baseline. **RMS: active**	
26	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect		
27	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect		
28	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28h	Uptake from the medium (in vitro)		No effect		
29	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28h	Uptake from the medium (in vitro)		No effect		
30	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28h	Uptake from the medium (in vitro)		No effect		
31	In vitro mechanistic	Thyroid receptor	Human liver cell line, HepG2	24h	Uptake from the medium (in vitro)		No effect		

ID	Effect classification	Effect target	Species / cell system	Duration of exposure	Route of administration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence (conclusions)
32	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	24h	Uptake from the medium (in vitro)		No effect		
33	In vitro mechanistic	Thyroid receptor	gene-proteins from E. coli, cell-free assay	0.5h	Uptake from the medium (in vitro)		No effect	**RMS:** CCTE_Simmons_quantiLum_inhib_2_dn (mono-oxygenase), Flags: <50% efficacy; only highest conc around baseline, borderline inactive.	
34	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect		
2	EATS-mediated	Thyroid weight	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		(3) In conclusion, T modality related adversity was not observed in a consistent manner for Metam, based on the absence of relevant thyroid weight or histopathological changes.
3	EATS-mediated	Thyroid weight	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
8	EATS-mediated	Thyroid weight	Rat	21d	Inhalation	1.54 mg/kg bw/day	**Decrease**	↓Absolute thyroid weight (no dose dependency observed). However, systemic toxicity (↓bw) and no histopathological changes in thyroid at this dose. Therefore, the ↓in thyroid weight is not considered related to T-mediated adversity but to general systemic toxicity.	
2	EATS-mediated	Thyroid histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
3	EATS-mediated	Thyroid histopathology	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
4	EATS-mediated	Thyroid histopathology	Rat	90d	Oral	>443 mg/kg bw/day	No effect		
6	EATS-mediated	Thyroid histopathology	Mouse	90d	Oral	>620 mg/kg bw/day	No effect		
8	EATS-mediated	Thyroid histopathology	Rat	21d	Inhalation	> 4.53 mg/L	No effect		
9	EATS-mediated	Thyroid histopathology	Rat	90d	Inhalation	> 160 mg/L	No effect		

ID	Effect classifi-cation	Effect target	Species / cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence (conclusions)
10	EATS-mediated	Thyroid histopathology	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
11	EATS-mediated	Thyroid histopathology	Rat	52-104wk	Oral	>190 mg/L	No effect		
12	EATS-mediated	Thyroid histopathology	Mouse	104wk	Oral	>230mg/L	No effect		

Assessment of each line of evidence THYROID modality for Metam: discussion

(1) There are only a few results available for **thyroid receptor**. While the molecular docking method (COSMOS) indicates low probability of binding, two models available in the Danish QSAR database yield a **positive result for metam sodium**, which is compliant with the applicability domain of the models. It can be related to the fact that the parent compound itself as well as structurally similar Metam Potassium (CAS No. 137-41-7) and methyldithiocarbamic acid (CAS No. 144-54-7) are present in the training sets of the models, and the experimental values are in their cases positive.

(2) **In vitro mechanistic effects** related to T modality were investigated in 12 screening assays ToxCast/EDSP21 with an overall negative (11/12 assays clearly inactive) outcome for T-related endocrine activity. One screening assay with the biological process target "regulation of catalytic activity" (enzyme activity) addressing TPO activity inhibition was considered positive. However, this assay result is of restricted reliability as the assay is flagged on the CompTox database as only 1 concentration is > the cut-off value and 50% efficacy is not reached. Moreover, metam is not metabolised to ethylene thiourea (ETU) which is the relevant metabolite and potent inhibitor of TPO of ethylene bis[dithiocarbamates] (EBDCs).

(3) Relevant adverse effects on thyroid *in-vivo* were not observed. Decreased thyroid weights were observed in one inhalation study with no corresponding histopathological changes. Moreover, general toxicity was observed at the same exposure concentration (significant reductions in bw). Therefore, the decrease in thyroid weight is not considered related to T-mediated adversity but to general systemic toxicity.

Assessment of the integrated lines of evidence THYROID modality for Metam: conclusions

(1)+(2)→ An activity for potential receptor binding was not observed in in silico and in vitro mechanistic data. Based on the available information an inhibition of the thyroperoxidase cannot be completely excluded but is considered unlikely. Overall, there is no evidence for endocrine activity with regards to the thyroid receptor based on in silico predictions and in vitro mechanistic data. A potential inhibitory effect on TPO cannot be excluded based on the available in silico and in vitro information.

3: In conclusion, there is no indication for endocrine-related adversity based on "EATS-mediated" parameters with regards to T modality based on in vivo repeated dose toxicity studies in rat, mouse, and dog.

2.1.2.2 MITC

Lines of evidence for the T-modality are included in **tables 2.1.2.2-1** (*in-silico, in-vitro*) and **2.1.2.2-2** (*in-vivo*).
RMS also revised and expanded the *in-vitro* data extracted from the EDSP database of the US EPA.
Details of the evaluation of the EDSP *in-vitro* studies (US EPA) are in Vol.3 B.6.8.3.

Assessment of the integrated lines of evidence THYROID for MITC (*in-silico* and *in-vitro*) modality: conclusions

An activity for potential receptor binding for MITC was not observed in a consistent way in neither *in silico* nor *in vitro* mechanistic data.

Assessment of the integrated lines of evidence THYROID modality for MITC (in-vivo): further discussion and conclusion

Changes in thyroid weight were observed in rat and mouse. However, these changes were not homogenous in effect direction as increases and decreases in thyroid weight were observed at similar dose levels. Moreover, corresponding histopathological effects were not observed and thyroid weight changes occurred at systemic toxic doses only (*e.g.* decreased body weight (gain)). Moreover, within chronic rat and mouse studies no thyroid-related tumours were observed. Therefore, T-mediated adversity was not observed based on potential effects on thyroid.
In conclusion, there is no indication for endocrine-related adversity based on «EATS-mediated» parameters with regards to T modality based on *in vivo* repeated dose toxicity studies in neither rat, mouse, or dog.

See tables 2.1.2.2-1 *(in-silico, in-vitro)* and 2.1.2.2-2 *(in-vivo)* below:

Table **2.1.2.2-1**: MITC Lines of evidence of THYROID modality: *in-silico* and *in-vitro* studies

ID	Effect classifi-cation	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
n.a.	In silico prediction	QSAR prediction T modality	n.a.	n.a.	n.a.	n.a.	**inconclusive** (applicability domain: out)	Danish QSAR DB: Thyroperoxidase (TPO) - MITC is present in the training set of the model and the experimental result is negative. The prediction is however inconclusive and the compound is considered to be outside the applicability domain of the model.	There are only a few results available for thyroid receptor. The molecular docking method (COSMOS) indicates low probability of binding. TPO inhibition was not observed for the training dataset. However, the QSAR model result is inconclusive as MITC is considered out of the applicability domain. **Summary conclusion on potential thyroid activity: Inconclusive**
n.a.	In silico prediction	QSAR prediction	n.a.	n.a.	n.a.	n.a.	No effect	COSMOS: THR: no potential NR ligand found; no indication for thyroid receptor activity	
26	In vitro mechanistic	Thyroid receptor	Human liver cell line, HepG2	24h	Uptake from the medium (in vitro)	9.85 µM	**Change**	ATG_THRa1_TRANS_up: observed borderline activity not considered relevant by the notifier Biological process target: regulation of transcription factor activity Flags: Hit-call potentially confounded by overfitting, Only one concentration > cut-off and one around cut off, therefore considered relevant by RMS. **RMS: Active**	In vitro mechanistic effects related to T modality were investigated in 13 screening assays ToxCast/EDSP21 with an overall negative (11/13 assays clearly inactive) outcome for T-related endocrine activity. **No <u>consistent</u> T-mediated endocrine activity was observed based on the available in vitro mechanistic data.**
27	In vitro mechanistic	Thyroid receptor	Human, cell-free	1h	Uptake from the medium (in vitro)	0.2 µM	**Change**	NVS_NR_hTRa_Antagonist: observed borderline activity not considered relevant by the notifier, due to lacking dose response, however an inverted U-shape response is possible in case of a biphasic response as a function of concentration. Biological process target: receptor binding Flags: Hit-call potentially confounded by overfitting, <50% efficacy; only one concentration > cut-off. **RMS: Active**	
28	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect	TOX21_TSHR_Agonist_ratio: no activity observed Biological process target: regulation of transcription factor activity	
29	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect	TOX21_TSHR_Antagonist_ratio: no activity observed Biological process target: regulation of transcription factor activity	

ID	Effect classifi-cation	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
30	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28h	Uptake from the medium (in vitro)		No effect	Tox21_TR_LUC_GH3_Agonist: no activity observed Biological process target: regulation of transcription factor activity	
31	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28	Uptake from the medium (in vitro)		No effect	Tox21_TR_LUC_GH3_Antagonist: no activity observed Biological process target: regulation of transcription factor activity	
32	In vitro mechanistic	Thyroid receptor	Rat pituitary gland cell line, GH3	28h	Uptake from the medium (in vitro)		No effect	Tox21_TR_LUC_GH3_Antagonist_viability: no activity observed Biological process target: cell proliferation	
33	In vitro mechanistic	Thyroid receptor	Human liver cell line, HepG2	24h	Uptake from the medium (in vitro)		No effect	ATG_THRa1_TRANS_dn: no activity observed Biological process target: regulation of transcription factor activity	
34	In vitro mechanistic	Thyroid receptor	Human kidney cell line, HEK293T	0.5h	Uptake from the medium (in vitro)		No effect	TOX21_TSHR_wt_ratio: no activity observed	

Table **2.1.2.2-2**: MITC Lines of evidence of THYROID modality: *in-vivo* studies

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
1	Thyroid weight	Dog	90d	Oral	>2 mg/kg bw/day	No effect		Changes in thyroid weight were observed in rat and mouse. However, these changes were not homogenous in effect direction as ↑ and ↓in thyroid weight were observed at similar dose levels. Moreover, corresponding histopathological effects were not observed and thyroid weight changes occured at systemic toxic doses only (e.g. ↓ body weight (gain)). Moreover, within chronic rat and mouse studies no thyroid-related tumours were observed. **Therefore, T-mediated adversity was not observed based on potential effects on thyroid.**
12	Thyroid weight	Rat	F0 & F1 adults	Inhalation	20 ppm	**Decrease**	Top-dose: significantly ↓mean absolute thyroid weight (♂F_0 & F_1, ♀F_1). However, while thyroid histopathology was not performed in the 2G study, no histopathological changes were observed in the chronic/ carcinogenicity study in rat. Moreover, ↓mean body weights and body weight changes were observed as indication for general systemic toxicity. Thus, the ↓thyroid weight is not considered relevant as regards ED effect.	
15	Thyroid weight	Mouse	78wk	Inhalation	15 ppm	**Increase**	Top-dose: significantly ↑relative (to b.w.) thyroid weights in ♂ which can be attributed to ↓final body weight. Moreover, no histopathological changes were observed and thus, the weight change is not considered toxicologically relevant.	
16	Thyroid weight	Rat	52-104wk	Inhalation	20 ppm	**Decrease**	significant ↓absolute and relative (to brain) thyroid weight in ♂ (104 weeks) only, which is considered to be a result of a MITC-related effect on final body weight; only top-dose effect. Moreover, at this dose level no histopathological changes were observed in the rat. In addition, ↓mean body weight (gain) was observed as a sign of general systemic toxicity. Based on mean body weights and cumulative body weights, the high-concentration of 20 ppm exceeded the maximum tolerated dose (MTD). Thus, thyroid weight change is not considered toxicologically relevant.	
17	Thyroid weight	Mouse	26-52-106wk	Oral	12 mg/kg bw/d	**Increase**	Week 52: The weight of the left thyroid gland (absolute + relative) significantly ↑ (♂, 80 + 200 ppm). The weight of the right thyroid gland significantly ↑ in ♀ at 80 ppm (relative only) and at 200 ppm. Week 104: Relative (to b.w.) thyroid weights significantly ↑ (200 ppm, ♀). No histopathological thyroid finding at any dose or sex, thus, thyroid weight change is not considered toxicologically relevant.	
18	Thyroid weight	Rat	53-104wk	Oral	1.6 mg/kg bw/d	No effect		
1	Thyroid histopathology	Dog	90d	Oral	>2 mg/kg bw/day	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
12	Thyroid histopathology	Rat	F0 & F1 adults	Inhalation	20 ppm	No effect	Thyroid histopathology was performed only in 2♀ (F_0: 20 ppm) and 1♂ (F_0, 5 ppm). No changes were observed.	
15	Thyroid histopathology	Mouse	78wk	Inhalation	>15 ppm	No effect		
16	Thyroid histopathology	Rat	52-104wk	Inhalation	>20 ppm	No effect		
17	Thyroid histopathology	Mouse	26-52-106wk	Oral	>26 mg/kg bw/d	No effect		
18	Thyroid histopathology	Rat	53-104wk	Oral	>1.6 mg/kg bw/day	No effect	No histopathological changes. C cell hyperplasia or in C-cell adenoma/carcinoma were observed but with poor dose-responsiveness, and not considered substance-related.	

2.1.2.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Metam
Relevant T-mediated adversity has not been observed in any of the repeated dose toxicity studies conducted with metam based on thyroid weight and histopathological examination. The only effect observed was reduced thyroid weight in a 21 day inhalation study in rats where also excessive general toxicity was observed (e.g. decreased body weight gain of at least 17%). Moreover, no corresponding histopathological changes were observed and no carcinogenic effect in thyroid was observed (details in Table 2.10-2 and LoE table).

T-mediated activity has been investigated in silico and in vitro. No indication for thyroid receptor (TR) activation is provided from in silico or in vitro data.
Thyroperoxidase inhibition cannot be excluded based on positive *in silico* and *in vitro* information. Positive *in vitro* data (NCCT data from EPA CompTox Database), which was also used as a training data set for the QSAR model, is of restricted reliability due to referenced limitations of the assay result (details in Table 2.10-3 and LoE table). However, ethylene thiourea (ETU), the relevant metabolite of ethylene bis[dithiocarbamates] (EBDCs) (formation presented in Figure 2.10-1) responsible for TPO inhibition, cannot be formed from metam based on its chemical structure as shown in Figure 2.10-2.

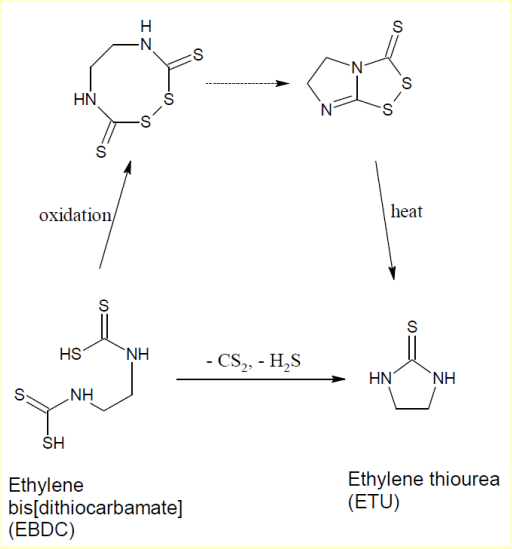


Figure 2.10-1: Generation of ETU from EBDC (adapted from EHC 78)[38]

[38] International Programme on Chemical Safety. Environmental Health Criteria 78. Dithiocarbamate Pesticides, Ethylenethiourea and Propylenethiourea: A General Introduction. Available at http://www.inchem.org/documents/ehc/ehc/ehc78.htm (accessed on 23 July 2019)

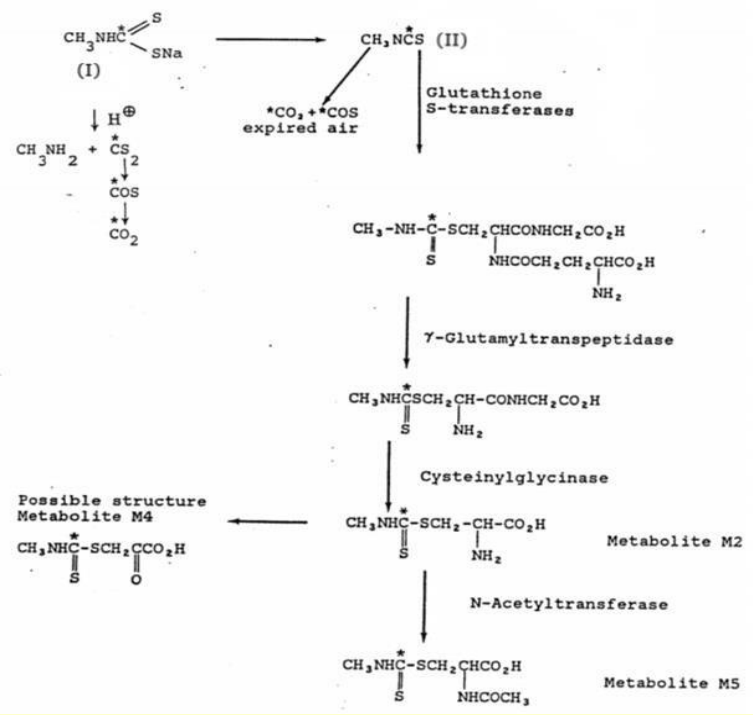


Figure 2.10-2: Metabolic pathway of metam sodium (I) and MITC (II) in the rat (for details refer to [redacted])

In conclusion, potential TPO inhibition of metam cannot be excluded based on the available *in silico* and *in vitro* data but is considered unlikely based on the substance's structure and metabolism. As no T-mediated adversity has been observed no further investigations on potential T-mediated endocrine activity are required.
However, to substantiate the weight of evidence approach for absence of T-related endocrine activity, **thyroid hormone measurements will be included in a Hershberger Assay (OECD TG 441)** if this study is considered necessary for investigation of potential EAS-related endocrine activity of metam.

RMS agrees with this assessment, with some comments. Obviously, a common metabolite of all dithiocarbamates, including metam, is carbon disulfide (CS_2), which may undergo further metabolism to form thiourea (recovered in urine as a major metabolite, EHC, 1979). Thioura has the thyroid as a target organ, which may partially explain the tendency of different dithiocarbamates to affect thyroid function. However, CS_2 is a very minor metabolite of metam and in view of the poor *in-vivo* effect of metam on the thyroid, unlike thiourea, the latter is unlikely to be formed at meaningful levels for this reason. On the other hand, CS_2 itself is of uncertain toxicity on the thyroid. The available human studies provide conflicting evidence on the adverse effects of CS_2 on thyroid function. These studies were limited by possible exposure to other chemicals, small sample size, and lack of quantification of precise exposure concentrations (ATSDR, 1996, Health Canada, 2000), and no *in-vivo* data are known in the animal for this endpoint.
Therefore, taking into account these uncertainties, **RMS** agrees with the submission of the proposed OECD TG441 study, extended with the assessment of thyroid parameters.

Cited references:

- *Environmental Health Criteria 10, Carbon disulfide, 1979.*
- *Toxicological profile for carbon disulfide, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), 1996.*
- *Priority substances list assessment report, Carbon Disulfide, Health Canada, 2000.*

Table 2.10-3: WoE for T-mediated adversity: Metam

- Changes in thyroid weight were observed in one study only (study ID 8) which is considered a result of general systemic toxicity. No effect on thyroid weight was observed in further RDT studies with dog (study ID 2, 3) and rabbit (study ID 10).
- Thyroid histological changes were not observed in RDT studies with rat (study ID 4, 8, 9, 11), mouse (study ID 6, 12), rabbit (study ID 10), and dog (study ID 2, 3).
- Carcinogenicity in thyroid was not observed in rat and mouse chronic/carcinogenicity studies (study ID 11, 12)
→ T-mediated adversity of metam is not observed.

Table 2.10-4: WoE for T-mediated endocrine activity: Metam

- in silico - Thyroid receptor (TR) binding: No TR activity has been predicted for metam
- in vitro - TR binding/activation: TR activity was not observed in 11 in vitro assays as referenced on the CompTox database (EPA)
- in silico - thyroperoxidase (TPO) inhibition: In silico investigations provided a positive indication for TPO inhibition by metam (applicability domain: in) based on a positive assay result of metam within the training data set of the QSAR model. This training data set is based on NCCT data of EPA (results described below).
- in vitro - TPO inhibition: One assay addressing TPO activity (CompTox database) showed a positive result for TPO inhibition by metam. However, this assay result is of restricted reliability as the assay is flagged on the CompTox database for the following reasons: only 1 concentration > cut-off value and 50% efficacy is not reached. Moreover, ethylene thiourea (ETU) is the relevant metabolite of ethylene bis[dithiocarbamates] (EBDCs) responsible for TPO inhibition. However, this metabolite cannot be formed based on the structure of metam.
→ Endocrine activity related to thyroid receptor binding of metam is not observed.

MITC

T-mediated adversity has not been observed in any of the repeated dose toxicity studies conducted with MITC based on thyroid weight and histopathological examination.

T-mediated activity has been investigated in silico and in vitro. No indication for thyroid receptor (TR) activation is provided from in silico or in vitro data.

No in vitro data on thyroperoxidase inhibition is available. However, no T-mediated adversity has been observed and thus, further investigations on potential T-mediated endocrine activity are not required. (For detailed information refer to Table 2.10-5, Table 2.10-6, and LoE table.)

Table 2.10-5: WoE for T-mediated adversity: MITC

- Changes in thyroid weight were observed in 4 studies (study ID 12, 15, 16, 17) with rat and mouse which are considered a result of general systemic toxicity not related to T-mediated adversity. No effect on thyroid weight was observed in further RDT studies with rat and dog (study ID 1, 18).
- No histopathological changes were observed in thyroid in RDT studies with rat (study ID 12, 16, 18), mouse (study ID 15, 17), and dog (study ID 1)
- Four carcinogenesis studies were conducted (2 each by oral and inhalation route). The MTD was exceeded in the inhalation studies at the top dose (for details refer to position statement on carcinogenicity).
→ T-mediated adversity of MITC is not observed.

Table 2.10-6: WoE for T-mediated endocrine activity: MITC

- in silico - Thyroid receptor (TR) binding: No TR activity has been predicted for MITC.
- in vitro - TR binding/activation: Endocrine activity related to T modality was investigated in 9 screening assays ToxCast/EDSP21 with an overall negative (7/9 assays clearly inactive) outcome for T-related endocrine activity.
- in silico - thyroperoxidase (TPO) inhibition: MITC is present in the training set of the model and the

experimental result is negative. The prediction is however inconclusive and the compound is considered to be outside the applicability domain of the model.
→ Endocrine activity related to thyroid receptor binding of MITC is not observed.

2.1.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Table 2.10-7: Selection of the relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	X (metam, MITC)
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.1.4 MoA analysis for T-modality

Not applicable.

2.1.4.1 Postulate MoA

Not applicable.

2.1.4.2. Further information to be generated to postulate MoA

Not applicable.

2.1.4.3. Empirical support of the postulated MoA

Not applicable.

2.1.4.4. Conclusion on MoA analysis

Not applicable.

2.1.5 Conclusion of the assessment of T-modality

Metam
T-mediated adversity of metam has been sufficiently investigated within 9 repeated dose toxicity studies (4 and 9 studies addressing thyroid weight and histopathology, respectively) with rat, mouse, rabbit, and dog. The only

effect observed was a decrease in thyroid weights in one subacute rat inhalation study (21 day exposure) with no corresponding histopathological changes. Therefore, no relevant T-mediated adverse effects were observed for metam.
With regards to T-mediated endocrine activity *in silico* and *in vitro* mechanistic data are available based on which there is no evidence that metam might interact with the thyroid receptor. Information on a potential inhibition of TPO is equivocal. However, ethylene thiourea (ETU), the relevant metabolite of ethylene bis[dithiocarbamates] (EBDCs) responsible for TPO inhibition, cannot be formed by metam based on its chemical structure.
According to the ED criteria laid down in Regulation (EU) 2018/605, endocrine mediated adversity as well as activity and the biological link between those two must be given to identify a substance as an endocrine disruptor. Since metam does not induce T-mediated adversity, which is sufficiently investigated based on a weight of the evidence approach, the ED criteria are not fulfilled (Scenario 1a, Table 2.10-7).
In conclusion, ED criteria for metam are not met with regards to T-modality.
However, if a Hershberger Assay (OECD TG 441) is considered necessary for investigation of A-related activity, thyroid hormone measurements will be included in the study design to substantiate the weight of evidence approach on T-related activity.

MITC
T-mediated adversity of MITC has been sufficiently investigated within 6 repeated dose toxicity studies with rat, mouse, rabbit, and dog. The only effect observed were changes in thyroid weight in repeated dose toxicity studies in mouse and rat. However, thyroid weight changes were not consistent and no corresponding histopathological changes were observed. Therefore, no relevant T-mediated adverse effects were observed for MITC.
With regards to T-mediated endocrine activity in silico and in vitro mechanistic data are available based on which there is no evidence that MITC might interact with the thyroid receptor. In silico information on potential inhibition of TPO is inconclusive.
According to the ED criteria laid down in Regulation (EU) 2018/605 for identification of a substance as an endocrine disruptor, the following criteria need to be fulfilled: identification of an adverse effect and endocrine activity as well as the biological link between those two.
MITC does not induce, which is sufficiently investigated based on a weight of the evidence approach, therefore, the ED criteria are not fulfilled (Scenario 1a, Table 2.10-7). In conclusion, the ED criteria for MITC are not met with regards to T-modality.

2.2 ED assessment for EAS-modalities

2.2.1 Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated
EAS-mediated parameters	**Metam** ***EAS-mediated adversity:*** Parameters for EAS-mediated adversity were investigated in repeated dose toxicity studies including a 2-Generation study according to OECD Guideline versions applicable at study performance (prior to 2001). As former OECD Test Guidelines did not address all ED relevant parameters as required in the ECHA/EFSA ED Guidance, the following parameters were <u>not investigated</u>: - age at vaginal opening - oestrus cyclicity - sperm parameters - age at balanopreputial separation - anogenital distance Therefore, EAS-mediated adversity is <u>not sufficiently investigated</u> for metam according to ECHA/EFSA ED Guidance. ***EAS-mediated activity:*** E: Output data from the ToxCast ER Bioactivity Model is available and thus, E-mediated activity is considered sufficiently investigated according to the ED Guidance.

	A: Output data from the ToxCast AR Bioactivity Model is available which is negative. However, according to the ED Guidance, the AR Bioactivity model is not sufficient for A-related activity investigation. Therefore, a tiered testing approach according to the ED Guidance is proposed including an Androgen Transactivation Assay (OECD TG 458) and a Hershberger Assay (OECD 441) if OECD TG 458 is negative. S: ToxCast/EDSP data on aromatase are available. For sufficiently investigating potential steroidogenic activity, the following tests according to chapter 3.4.1 of the ECHA/EFSA ED Guidance are proposed: H295R steroidogenesis assay (OECD TG 456) and aromatase assay (human recombinant, OPPTS 890.1200) **MITC** ***EAS-mediated adversity:*** Parameters for EAS-mediated adversity were investigated in repeated dose toxicity studies including two two-generation reproductive toxicity studies according to OECD Guideline versions applicable at study performance. One 2-Generation study was performed according to OECD TG 416 (2001) investigating all relevant EATS-related parameters as referenced in the ECHA/EFSA Guidance (Table 14). Therefore, EAS-mediated adversity of MITC is sufficiently investigated according to ECHA/EFSA ED Guidance. ***EAS-mediated activity:*** E: Output data from the ToxCast ER Bioactivity Model is available and thus, E-mediated activity is considered sufficiently investigated. A: Output data from the ToxCast AR Bioactivity Model is available which is negative. According to the ECHA/EFSA ED Guidance, Arelated endocrine activity is not sufficiently investigated. However, since adversity was not observed, although "sufficiently investigated" according to chapter 3.4.1 of the ECHA/EFSA ED Guidance, no further tests addressing A-related endocrine activity are considered necessary. S: ToxCast/EDSP data on aromatase are available which are negative. According to the ECHA/EFSA ED Guidance the tests as referred to in chapter 3.4.3 Table 4 (H295R steroidogenesis assay (OECD TG 456) and aromatase assay (human recombinant, OPPTS 890.1200)) need to be available for sufficient S-related activity investigation. However, since EAS-mediated adversity was not observed although considered "sufficiently investigated" according to chapter 3.4.1 of the ECHA/EFSA ED Guidance, no further testing for S-related endocrine activity of MITC is required.

2.2.2 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

2.2.2.1 Metam

Lines of evidence for the EAS-modalities of Metam are included in **table 2.2.2.1-1** through **2.2.2.1-5 .**
RMS also revised and expanded the *in-vitro* data extracted from the EDSP database of the US EPA.
Details of the evaluation of the EDSP *in-vitro* studies (US EPA) are in Vol.3 B.6.8.3.

See tables 2.2.2.1-1 through 2.2.2.1-5 below:

Table **2.2.2.1-1**: Metam Lines of evidence: EAS modalities, *in-silico* and *in-vitro* studies

ID	Effect classification	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
n.a.	In silico prediction	Androgen receptor (A-modality)	n.a.	n.a.	n.a.	n.a.	No effect	(1) none	(1) no androgenic activity.
20	In vitro mechanistic	Androgen receptor (A modality)	-			0 µM	No effect	(2) none	(2) no androgenic activity.
-*	In vitro mechanistic	Androgen receptor (A modality)	ovary cell line, CHO-K1	24h	Uptake from the medium (in vitro)	0.962 µM	**Change**	**RMS:** OT_AR_ARELUC_AG_1440 ↑at low concentrations, ↓at high concentrations, >cut-off	equivocal
-*	In vitro mechanistic	Androgen receptor (A modality)	Human kidney cell line, HEK293T	16h			No effect	RMS: OT_AR_ARSRC1_0960: only ↑top-concentration but <cut-off	equivocal
n.a.	In silico prediction	Oestrogen receptor (E-modality)	n.a.	n.a.	Uptake from the medium (in vitro)	n.a.	No effect	(3) none	(3) no oestrogenic activity.
19	In vitro mechanistic	Oestrogen receptor (E-modality)	-			0 µM	No effect	(4) none	(4) no oestrogenic activity.
-* -* -*	In vitro mechanistic	Oestrogen receptor (E-modality)	Human kidney cell line, HEK293T	24h	Uptake from the medium (in vitro)		No effect	**RMS:** OT_ER_ERαERβ_1440 OT_ER_ ERαERβ_1440 OT_ER_ ERβERβ_1440 only ↑top-concentrations but <cut-off; 8h timepoint: inactive	equivocal
-*	In vitro mechanistic	Oestrogen receptor (E-modality)	Human breast cell line, VM7	22h	Uptake from the medium (in vitro)		No effect	**RMS :** TOX21_ERa_ LUC_VM7_Agonist High-variable concentration response, with only ↑top-concentration (100 µM) around cut-off; it is of note that the main metabolite MITC displays the same behaviour in this assay.	equivocal

ID	Effect classification	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
n.a.	In silico prediction	Steroidogenesis (S-modality)	n.a.	n.a.	n.a.	n.a.	No effect	(5) none	(5) no steroidal activity.
21	In vitro mechanistic	CYP19 (S-modality)	Human breast cell line, MCF-7	24h	Uptake from the medium (in vitro)	0 µM	No effect	(6) none	(6+7) no steroidal activity.
22	In vitro mechanistic	CYP19 (S-modality)	Human breast cell line, MCF-7	24h	Uptake from the medium (in vitro)	0 µM	No effect	(7) none	

*: assays present in the ToxCast database, but not highlighted by the notifier (hence no Id identifier in ED report).

Assessment of each line of evidence and integrated lines of evidence EAS modality (*in-silico* and *in-vitro*): further discussion and conclusions

Observed effect (+ and -)	Assessment of each line of evidence	Assessment on the integrated line of evidence
(1) Due to the quality of COMPARA consensus predictions in combination with other models (predicting no androgenic activity), the assessment of androgenic activity based on the available models is considered reliable.	(1) The available in silico data provides supporting evidence that the a.s. has no androgenic activity.	**(1+2)** Only sporadic (equivocal) effects seen at top-concentrations or with unexplained concentration-response effects. In-vitro assays overall negative. **In conclusion, there is no indication for endocrine activity with regards to A modality.**
(2) ToxCast prediction model is negative. Thus no hint for endocrine activity with regards to A modality is observed based on in vitro mechanistic data.	(2) Based on the results of the ToxCast AR prediction model no indication for androgenic activity was observed.	
(3) due to the high amount of data available on ER activity and the high quality of CERAPP Consensus predictions the assessment of ER activity is considered reliable. The CERAPP consensus predictions coincide well with the output of the AUC model which are both negative.	(3) The available in silico data provides supporting evidence that the a.s. has no oestrogenic activity.	**(3+4)** Only sporadic (equivocal) effects seen at top-concentrations or with unexplained concentration-response effects. In-vitro assays overall negative. **In conclusion, there is no indication for endocrine activity with regards to E modality.**
(4) ToxCast prediction model is negative. Thus no indication for endocrine activity with regards to E modality is observed based on in vitro mechanistic data.	(4) Based on the results of the ToxCast ER prediction model no indication for oestrogenic activity was observed.	
(5) Only one model is available for steroid receptors: glucocorticoid receptor (GR). Steroidal activity is evaluated by means of the molecular docking method (COSMOS). The results indicate low probability of binding.	(5) The available in silico data provides supporting evidence that the a.s. has no steroidal activity. It should be remarked that only 1 in-vitro assay (TOX21_Aromatase_ Inhibition) was performed (along with its cell viability counterpart).	**(5+6+7) In conclusion, there is no indication for endocrine activity with regards to S modality.**
(6) TOX21_Aromatase_Inhibition assay is inactive.	(6+7) The available in vitro mechanistic data based on results of the CompTox Database are negative. Thus, there is no indication for endocrine activity (S modality) based on in vitro mechanistic data.	
(7) TOX21_Aromatase_Inhibition_viability assay is inactive.		

Table **2.2.2.1-2**: Metam Lines of evidence: EAS mediated, *in-vivo* studies, males

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
2	Epididymis weight	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect	none	(1+2+3) Epididymides weight was decreased in a subchronic dog and a chronic mouse study at the high dose level. However, in the dog general toxicity was observed at the same dose level (dog: mortality, decreased bw and fc, changes in clinical chemistry parameters; mouse: decreased bw). No corresponding histopathological changes were observed in the dog, and slight epididymis mononuclear cell infiltration at top-dose was observed in the chronic mouse study. He latter is of uncertain relevance as regards endocrine adversity. **In conclusion, no EAS-mediated adversity with respect to effects on epididymides was observed, however the findings are seen in the presence of systemic toxicity.**
3	Epididymis weight	Dog	90d	Oral	10 mg/kg bw/day	**Decrease**	(1) n.s.s. ↓Absolute organ weight, no effect on relative organ weight. In the absence of pathological changes, the effect is not considered EAS-related.	
12	Epididymis weight	Mouse	104wk	Oral	7 mg/kg bw/day	**Decrease**	(2) significant ↓absolute (7mkd) and relative (29mkd) organ weight. Slight changes in the mouse epididymis (see 3).	
18	Epididymis weight	Rat	11wk	Oral	>100 mg/L water	No effect	none	
2	Epididymis histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect	none	
6	Epididymis histopathology	Mouse	90d	Oral	>620 mg/L	No effect	none	
9	Epididymis histopathology	Rat	90d	Inhalation	> 0.16	No effect	none	
11	Epididymis histopathology	Rat	52-104wk	Oral	190 mg/L	No effect	none	
12	Epididymis histopathology	Mouse	104wk	Oral	29 mg/kg bw/day	No effect	(3) ↑of total epididymis mononuclear cell infiltration at top-dose. The increase is slight (7/55) *vs.* ctrl (3/55). WBC ilfiltration in epididymis is not considered EAS-related.	
2	Testis weight	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect	none	(4+5+6+7) A decrease in testis weight was observed in rat and dog after 21 and 90 days of exposure, respectively. Moreover, inhibited spermatogenesis was observed in the rat (21 day exposure). However, these changes are considered secondary to observed general toxicity such as reduced body weight changes and increased mortality (rat only). In addition, in chronic oral studies in dog and rat no effects on testis were observed. In the chronic mouse study,

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
3	Testis weight	Dog	90d	Oral	10 mg/kg bw/day	**Decrease**	(4) Sign. ↓absolute organ weight. The same trend was observed for relative organ weight, however, no statistical significance was observed. In the absence of pathological changes and general toxicity (↓body weight) observed, the effect is not considered EAS-related.	a slight incidence of moderate testis atrophy was limited to the top-dose. Therefore, no coherence in time relating to potential effects in testis was observed, and when observed it was confounded by meaningful systemic toxicity. **In conclusion, EAS-mediated adversity with respect to effects on testes was not observed.**
4	Testis weight	Rat	90d	Oral	27 mg/kg bw/day	No effect	none	
6	Testis weight	Mouse	90d	Oral	>79 mg/kg bw/day	No effect	none	
7	Testis weight	Rat	21d	Oral	>27 mg/kg bw/day	No effect	none	
8	Testis weight	Rat	21d	Inhalation	1.54 mg/L	**Decrease**	(5) ↓Testis weight at 1.54 mg/L (-2-3%) and (-19%). However, at top-dose ↑mortality (10/25 animals) and signs of general toxicity such as ↓body weight gain (-25%) and changes in haematological parameters were also reported for the mid dose group (1.54 mg/L) and above. Therefore, changes in testis weight are secondary to general toxicity.	
10	Testis weight	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect	none	
11	Testis weight	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect	none	
12	Testis weight	Mouse	104wk	Oral	> 29 mg/kg bw/day	No effect	none	
18	Testis weight	Rat	11wk	Oral	>100 mg/L water	No effect	none	
2	Testis histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect	none	
3	Testis histopathology	Dog	90wk	Oral	> 10 mg/kg bw/day	No effect	none	
4	Testis histopathology	Rat	90d	Oral	>443 mg/L water	No effect	none	
6	Testis histopathology	Mouse	90d	Oral	>620 mg/L water	No effect	none	

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
8	Testis histopathology	Rat	21d	Inhalation	4.53 mg/L	**Change**	(6) ↓spermatogenesis in the testicle in 8/25 at top-dose. However, at this dose level ↑mortality (10/25) and further signs of general toxicity (↓body weight, fc, changes in haematological parameters) were observed at the top-dose. Therefore, the observed changes in spermatogenesis are considered secondary to general toxicity.	
9	Testis histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect	none	
10	Testis histopathology	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect	none	
11	Testis histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect	none	
12	Testis histopathology	Mouse	104wk	Oral	29 mg/kg bw/day	**Change**	(7) ↑of moderate testis atrophy at top-dose. The increase is slight (5/55) *vs.* ctrl (0/55).	
6	Seminal vesicles histopathology	Mouse	90d	Oral	>620 mg/L	No effect	none	
9	Seminal vesicles histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect	none	
11	Seminal vesicles histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect	none	
12	Seminal vesicles histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect	none	
18	Seminal vesicles histopathology	Rat	11wk	Oral	>100 mg/L water	No effect	none	
2	Prostate histopathology*	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect	none	
3	Prostate histopathology*	Dog	90d	Oral	> 10 mg/kg bw/day	No effect	none	
4	Prostate histopathology*	Rat	90d	Oral	27 mg/kg bw/day	No effect	none	
6	Prostate histopathology*	Mouse	90d	Oral	>620	No effect	none	
8	Prostate histopathology*	Rat	21d	Inhalation	4.53 mg/L	**Change**	(8) Prostate atrophy was observed (9/25 *vs* 0/25). However, at this dose level increased mortality (10/25) and further signs of general toxicity were observed (*e.g.* ↓body weight, clinical signs, changes in haematological parameters).	(8) Prostate atrophy was observed in 1 subacute study in rats. However, in further subchronic and chronic studies no effects on prostate were observed and thus, coherence in time relating to potential effects on prostate was not evident.

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
								In conclusion, EAS-mediated adversity with respect to effects on prostate was not observed.
9	Prostate histopathology*	Rat	90d	Inhalation	> 0.16 mg/L	No effect	none	
11	Prostate histopathology*	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect	None (error in notifier's file: no change)	
12	Prostate histopathology*	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect	none	
18	Prostate histopathology*	Rat	11wk	Oral	>100 mg/L water	No effect	none	

*: including seminal vesicles and coagulating glands;
Remark RMS: Study ID8 (21d inhalation study, [redacted] 1979: The NOAEL < 0.51 mg/L breathing air (lowest concentration, based upon ↓body weight on d21. However, this value is not reliable due to methodology flaws and the presence of a better study conducted with longer duration.)

Table **2.2.2.1-3**: Metam Lines of evidence: EAS mediated, *in-vivo* studies, females

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
8	Ovary weight	Rat	21d	Inhalation	1.54 mg/L	**Decrease**	(1) significant ↓ ovary weight (-18.0% at 1.54 mg/L and -42.0% at 4.53 mg/L)	(1+2) Changes in ovary weight were observed in rat inhalation studies. However, the effect was not consistent as a decrease was observed in the subacute study and an increase in relative weight was observed in the subchronic study. Moreover, no histopathological changes were observed in subchronic and chronic studies.
9	Ovary weight	Rat	90d	Inhalation	0.16 mg/L	**Increase**	(2) significant ↑ in relative ovary weight (+31%)	
10	Ovary weight	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
2	Ovary histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
4	Ovary histopathology	Rat	90d	Oral	27 mg/kg bw/day	No effect		
6	Ovary histopathology	Mouse	90d	Oral	>620 mg/L	No effect		
9	Ovary histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
10	Ovary histopathology	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
11	Ovary histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Ovary histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
13	Uterus weight*	Rat	GD7-16	Oral	> 20 mg/kg bw/day	No effect		(3+4+5+6) A decrease in gravid uterus weight was observed in developmental studies in the rabbit starting from 40 mg/kg bw/d which is linked to the reduced litter size and maternal toxicity. Overall no histopathological adverse effect was observed in repeated dose toxicity studies in rat, mouse, and dog.
15	Uterus weight*	Rat	GD7-15	Oral	> 20 mg/kg bw/day	No effect		
16	Uterus weight*	Rabbit	13 (GD 6-18)	Oral	100 mg/kg bw/day	**Decrease**	(3) ↓ in gravid uterus weight	
17	Uterus weight*	Rabbit	13 (GD 8-20)	Oral	60 mg/kg bw/day	**Decrease**	(4) ↓ in uterus weight	
37	Uterus weight*	Rabbit	13 (GD 8-20)	Oral	40 mg/kg bw/day	**Decrease**	(5) ↓ in gravid uterus weight 40 and 60 mg/kg bw/day dose group, reaching statistical significance at 60 mg/kg bw/day.	
2	Uterus histopathology*	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
4	Uterus histopathology*	Rat	90d	Oral	27 mg/kg bw/day	No effect		
6	Uterus histopathology*	Mouse	90d	Oral	>620	No effect		
8	Uterus histopathology*	Rat	21d	Inhalation	> 4.53 mg/L	No effect		

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
9	Uterus histopathology*	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
11	Uterus histopathology*	Rat	52-104wk	Oral	13 mg/kg bw/day	**Change**	(6) slight ↑ Incidence of total glandular dilatation	
12	Uterus histopathology*	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
9	Vagina histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
11	Vagina histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
2	Cervix histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
6	Cervix histopathology	Mouse	90d	Oral	>620 mg/L water	No effect		
9	Cervix histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
11	Cervix histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Cervix histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
2	Mammary gland histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
3	Mammary gland histopathology	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
6	Mammary gland histopathology	Mouse	90d	Oral	>620 mg/L water	No effect		
8	Mammary gland histopathology	Rat	21d	Inhalation	> 4.53 mg/L	No effect		
9	Mammary gland histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
11	Mammary gland histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Mammary gland histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
9	Mammary gland histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		

*: including cervix

Remark RMS: Study ID8 (21d inhalation study, [redacted] 1979: The NOAEL < 0.51 mg/L breathing air (lowest concentration, based upon ↓body weight on d21. However, this value is not reliable due to methodology flaws and the presence of a better study conducted with longer duration.)

Assessment of each line of evidence and integrated lines of evidence EAS modality (*in-vivo*) : further discussion and conclusions

In the **males**, occasional epididimydes and testes weight drops were observed, in the presence or in the absence of histopathological findings. Prostate atrophy was observed in a suboptimal 21d inhalation rat study, not further considered in the toxicological package, and prostate findings were absent in other rat studies. There was no coherence in time relating to potential effects in testis, epididymis, and prostate, and when observed it was confounded by meaningful systemic toxicity.
In addition, the findings are not corroborated by in-vitro or in-silico observations, and overall the effects are more related to concomitant systemic toxicity than to plausible endocrine mechanisms.
In the **females**, changes in ovary weight were observed in rat inhalation studies. However, the effect was not consistent as a decrease was observed in the subacute study and an increase in relative weight was observed in the subchronic study. Moreover, no histopathological changes were observed in subchronic and chronic studies.
A decrease in gravid uterus weight was observed in developmental studies in the rabbit starting from 40 mg/kg bw/d which is linked to the reduced litter size and maternal toxicity. Overall no histopathological adverse effect was observed in repeated dose toxicity studies in rat, mouse, and dog.
No adverse effects on neither vagina and cervix histopathology, nor mammary gland histopathology were observed.
RMS considers that no coherent EAS-mediated adversity with respect to effects on ovaries, uterus, vagina, cervix, nor mammary gland was identified in the toxicity studies assessing repeated administration.
In conclusion, RMS considers that there is no indication for endocrine-related adversity based on "EATS-mediated" parameters in neither the males nor the females.

Table 2.2.2.1-4: Metam Lines of evidence: sensitive to, but not diagnostic of EATS, *in-vivo* studies.

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
8	Pituitary weight	Rat	21d	Inhalation	1.54 mg/L	**Decrease**	↓absolute pituitary weight (-25%♂, -30%♀ at 1.54 mg/L and -38%♂, -40%♀ at 4.53 mg/L). However, no histopathological changes were observed at these inhalation concentrations.	Relevant adverse effects on pituitary were not observed. Decreases in pituitary weight observed in an inhalation studies did not correspond to histopathological changes. Moreover, general toxicity was observed at the same exposure concentration level (significant reductions in bw). **In conclusion, adversity sensitive to but not diagnostic of T modality was not observed for Metam sodium based on the absence of relevant pituitary weight or histopathological changes.**
10	Pituitary weight	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
2	Pituitary histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
3	Pituitary histopathology	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
6	Pituitary histopathology	Mouse	90d	Oral	>620 mg/L water	No effect		
8	Pituitary histopathology	Rat	21d	Inhalation	> 4.53 mg/L	No effect		
9	Pituitary histopathology	Rat	90d	Inhalation	> 160 mg/L	No effect		
10	Pituitary histopathology	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
11	Pituitary histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Pituitary histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
2	Adrenals weight	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		Some changes in adrenal weight were observed in rat only. These changes were not consistent (*i.e.* ↑and ↓observed) and assessed as a result secondary to general toxicity. Moreover, no histopathological changes were observed. **In conclusion, metam sodium does not induce adverse effects on adrenals.**
3	Adrenals weight	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
4	Adrenals weight	Rat	90d	Oral	27 mg/kg bw/day	**Decrease**	↓absolute adrenal weight. However, this effect is considered secondary to general toxicity as ↓body weight at 8.1 mg/kg bw/d onwards. Moreover, no histopathological changes were observed.	
6	Adrenals weight	Mouse	90d	Oral	>620 mg/L water	No effect		
8	Adrenals weight	Rat	21d	Inhalation	1.54 mg/L	**Decrease**	↓significant absolute adrenal weight. (-14%♀. However, at this dose level ↑mortality (10/25♂; 1/25♀) and further signs of general toxicity were observed (e.g. ↓body weight, clinical signs, changes in haematological parameters).	
9	Adrenals weight	Rat	90d	Inhalation	0.16 mg/L	**Increase**	↑relative adrenal weight, possibly attributed to a ↓body weight observed at this dose level. Moreover, no histopathological changes were observed.	
10	Adrenals weight	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
11	Adrenals weight	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Adrenals weight	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		
2	Adrenals histopathology	Dog	52wk	Oral	> 1 mg/kg bw/day	No effect		
3	Adrenals histopathology	Dog	90d	Oral	> 10 mg/kg bw/day	No effect		
4	Adrenals histopathology	Rat	90d	Oral	>443 mg/L water	No effect		
6	Adrenals histopathology	Mouse	90d	Oral	>620 mg/L water	No effect		
9	Adrenals histopathology	Rat	90d	Inhalation	> 0.16 mg/L	No effect		
10	Adrenals histopathology	Rabbit	21d	Dermal	> 125 mg/kg bw/day	No effect		
11	Adrenals histopathology	Rat	52-104wk	Oral	>13 mg/kg bw/day	No effect		
12	Adrenals histopathology	Mouse	104wk	Oral	>29 mg/kg bw/day	No effect		

Remark RMS: Study ID8 (21d inhalation study, [redacted] 1979: The NOAEL < 0.51 mg/L breathing air (lowest concentration, based upon ↓body weight on d21. However, this value is not reliable due to methodology flaws and the presence of a better study conducted with longer duration.)

Note: parameters STBNDO in table 2.2.2.1-4 are potentially related to both T- and EAS modalities.

Assessment of the integrated line of evidence of findings «sensitive to but not diagnostic of EATS» (*in-vivo*) in hormone-sensitive organs and tissues: further discussion and conclusions

Changes in pituitary and adrenals were not consistent and assessed as a result secondary to general toxicity. It should be noted that adversity in the adrenal and pituitary can be consequent to disruption of the HPA-axis resulting in altered stress response, which is likely in the case of metam.

In conclusion, there is no indication for endocrine-related adversity based on «sensitive to but not diagnostic of EATS» parameters in pituitary and adrenal glands, based on *in vivo* repeated dose toxicity studies in rat, mouse, and dog.

Table **2.2.2.1-5**: Metam Lines of evidence: sensitive to, but not diagnostic of EAS, *in-vivo* studies.

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
15	Fertility	Rat	10d (GD 6-15)	Oral	>120 mg/kg bw/day	No effect	Conception rate and incidence of abortions were not affected by treatment.	**No EAS-related adversity was observed with regards to fertility parameters.**
18	Fertility	Rat	11d	Oral	>12 mg/kg bw/day	No effect	The number of pregnancies as well as abortions was not affected.	
37	Fertility	Rabbit	13d (GD 8-20)	Oral	>60 mg/kg bw/day	No effect	The number of pregnancies as well as abortions was not affected.	
14	Foetal development	Rat	10d (GD 7-16)	Oral	5 mg/kg bw/day	**Change**	↑unossified odontoid, cervical vertebrae and calcaneum (variants); a minor defect in a cervical vertebrae center. ≥20 mg/kg/d: ↓foetal weight, ↑several vertebral column ossification delays. Top-dose: ↑post-implantation losses, intra-uterine deaths, 1 unossified cervical arches, 2/2 microphthalmia, 1 anophthalmia, 1 short upper jaw/cleft lip, 3/3 internal hydrocephaly, 1 cerebral meningocoele, ↑manus/pes scores (↓ossification).	Some effects of metam sodium on foetal development with regards to retarded ossification were observed. Isolated finding of decreased placental weight in rats (increased in rabbits), but not investigated in most studies. The latter is unlikely related to EAS, but rather with hypoxia/GSH depletion, the latter overtly affected by both metam and MITC. Overall, effects attributed to general foetotoxicity and/or maternal toxicity. In conclusion, **no adverse EAS-related effect on foetal development was observed.**
15	Foetal development	Rat	10d (GD 6-15)	Oral	10 mg/kg bw/day	**Change**	↑dose-related incidence of skeletal variations and retardations (ossification delays). ≥40 mg/kg/d: ↓foetal weight gain, ↓placental weight Top-dose: 2/1 meningocoele, 1 bilateral microphthalmia.	
16	Foetal development	Rabbit	13 (day 6-18)	Oral	10 mg/kg bw/day	**Change**	↑post-implantation loss, intra-uterine deaths, number of dead implants, ↑visceral variations ≥30 mg/kg/d: gall bladder agenesis, asymetrical sternebrae. Top-dose: ↑truncus arteriosus communis, 1 spina bifida, 1 meningocoele, Notifier considers the compound-related increase of skeleton variations in this group sign of «*immaturity of the foetuses*». Overall, RMS agrees that most effect are accountable to maternotoxic doses.	
17	Foetal development	Rabbit	13 (GD 8-20)	Oral	20 mg/kg bw/day	**Change**	↑minor skeletal defects, ↓ossification Top-dose: ↓foetal weight, ↑post-implantation losses, intra-uterine deaths, 1 cerebral meningocoele, 2/2 cleft palate.	
18	Gestation length	Rat	11	Oral	>12 mg/kg bw/day	No effect		**No EAS-related adversity was observed with regards to gestation length.**
18	Gestation length	Rat	11	Oral	>12 mg/kg bw/day	No effect		

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
13	Litter size	Rat	GD 7-16	Oral	> 20 mg/kg bw/day	No effect		**No EAS-related adversity was observed with regards to litter size.**
18	Litter size	Rat	11	Oral	>12 mg/kg bw/day	No effect		
18	Litter viability	Rat	11	Oral	>12 mg/kg bw/day	No effect	No adverse effect on litter viability was observed in the 2 generation study.	**No EAS-related adversity was observed with regards to litter viability.**
13	Litter/ pup weight	Rat	GD 7-16	Oral	80 mg/kg bw/day	No effect		The reduced litter/pup weight is attributed to general foetotoxicity and/or maternal toxicity. Therefore, **the reduced litter/pup weight is not considered to be based on EAS-related adversity**.
14	Litter/pup weight	Rat	10d (GD 7-16)	Oral	20 mg/kg bw/day	**Decrease**	Significant ↓foetal weight at the mid (-5%) and top (15%) dose. Based on general maternal toxicity (↓ body weight >10%, ↓fc >20%), salivation, stain around mouth, urinary incontinence, vaginal bleeding, kidney pelvic dilatation, the MTD was exceeded.	
15	Litter/pup weight	Rat	10d (GD 6-15)	Oral	40 mg/kg bw/day	**Decrease**	↓Foetal mean weight gain at ≥40 mg/kg bw/day. Clear evidence of maternal toxicity was seen at 40 and 120 mg/kg/day dose levels of metam-sodium as shown by dose-related ↓bodyweight, bodyweight gain (120 mg/kg bw/d: 17-183%) and fc (120 mg/kg bw/d: 15-19%) during the dosing period.	
16	Litter/pup weight	Rabbit	13d (day 6-18)	Oral	>100 mg/kg bw/day	No effect		
17	Litter/pup weight	Rabbit	13d (GD 8-20)	Oral	60 mg/kg bw/day	**Decrease**	↓Foetal weight but already at a dose of 20 mg/kg bw/d maternal animals showed signs of general toxicity (↓body weight gain and ↓fc).	
18	Litter/pup weight	Rat	11d	Oral	12 mg/kg bw/day	**Decrease**	↓pup/litter weight in both sexes and both generations (10-15%). F_1 litter: ↓litter weights at ≥4 mg/kg bw/day at d22 and d29 but these differences were not statistically significant. ↓bw in both sexes of F_0/F_1 parents. About 5% in both sexes up to week 11, and about 10% in ♀ during pregnancy. Moreover, ↓bw during lactation.	

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
37	Litter/pup weight	Rabbit	13d (GD 8-20)	Oral	40 mg/kg bw/day	**Decrease**	↓Litter and foetal weight at 40 mg/kg bw/day and above, reaching statistical significance at 60 mg/kg bw/day for litter weight only. Significant ↓maternal body weight gain at 20 mg/kg bw/day and above: ↓faecal output, ↓body weight gain, ↓fc Top-dose 60 mg/kg bw/day: ↑sloughed mucosa glandular stomach.	
15	# implantations, corpora lutea	Rat	10d (GD 6-15)	Oral	>120 mg/kg bw/day	No effect		Adverse effect on the number of implantations in both rats and rabbits, but mostly attributed to general foetotoxicity and/or maternal toxicity. Therefore, **the reduced litter/pup weight is not considered to be based on EAS-related adversity**.
16	# implantations, corpora lutea	Rabbit	13d (day 6-18)	Oral	10 mg/kg bw/day	**Decrease**	↑post-implantation loss, intra-uterine deaths, number of dead implants	
17	# implantations, corpora lutea	Rabbit	13 (GD 8-20)	Oral	60 mg/kg bw/day	**Decrease**	Top-dose: ↓foetal weight, ↑post-implantation loss, intra-uterine deaths	
18	# implantations, corpora lutea	Rat	11d	Oral	>12 mg/kg bw/day	No effect		
13	# embryonic or foetal deaths & viable foetuses	Rat	10d (GD 7-16)	Oral	20 mg/kg bw/day	**Change**	↓foetal weight, ↑intra-uterine deaths	The reduced number of live foetuses is attributed to general foetotoxicity and/or maternal toxicity, where also ↑in post-implantation loss was recorded. Therefore, **the reduction in live foetuses is not considered to be based on EAS-related adversity.**
15	# embryonic or foetal deaths & viable foetuses	Rat	10d (GD 6-15)	Oral	10 mg/kg bw/day	**Change**	↓live foetuses (%/animal): significant at low and high dose (82%, 85%) but not at mid dose (93%) when compared to control (93%). No dose response, thus RMS agrees that the finding is unconclusive in this study. However, embryonic death is found in other studies so it cannot be ignored overall.	
16	# embryonic or foetal deaths & viable foetuses	Rabbit	13 (GD 6-18)	Oral	10 mg/kg bw/day	**Change**	At 10 mg/kg bw/day ↑post-implantation loss*, intra-uterine deaths, number of dead implants, attaining significance* (or more meaningful↑) at ≥30 mg/kg bw/day. Maternal toxicity (↓body weight) at ≥30 mg/kg bw/day.	
17	# embryonic or foetal deaths & viable foetuses	Rabbit	13 (GD 8-20)	Oral	60 mg/kg bw/day	**Change**	At top-dose 60 mg/kg bw/day: ↓foetal weight, ↑post-implantation losses, intra-uterine deaths (↓live foetuses *in utero* at study termination). At ≥20 mg/kg bw/day maternal animals showed general toxicity (↓body weight and body weight gain).	

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
37	# embryonic or foetal deaths & viable foetuses	Rabbit	13 (GD 8-20)	Oral	40 mg/kg bw/day	**Increase**	Slight ↑intra uterine deaths at 40 mg/kg bw/d attaining significance at top-dose of 60 mg/kg bw/day, with dose-response but in the presence of significant ↓maternal body weight gain, ↓fc and ↑clinical signs from 20 mg/kg bw/day onwards over the study period.	
18	# live births	Rat	11	Oral	>12 mg/kg bw/day	No effect		**No EAS-related adversity was observed with regards to the number of live births.**
13	Post implantation loss	Rat	10 (GD 7-16)	Oral	20 mg/kg bw/day	**Increase**	↓foetal weight, ↑post-implantation loss, intra-uterine deaths. It is noted that the ↑PIL lacks dose-responsiveness in this pilot study, but in the light of comparable effects in other developmental studies, involvement of treatment cannot be ignored.	The increase in post implantation loss is attributed to general foetotoxicity elicited by maternal toxicity observed at foetotoxic doses. Therefore, the increase in **post implantation loss is not considered to be based on EAS-related adversity.**
15	Post implantation loss	Rat	10 (GD 6-15)	Oral	10 mg/kg bw/day	**Increase**	Significant ↑PIL at low and top dose (~18%, 15%) but <u>not</u> at mid dose (7%) when compared to control (7%): no dose response. Clear evidence of maternal toxicity at ≥40 mg/kg/day: dose-related ↓bodyweight, bodyweight gain (120 mg/kg bw/d: 17-183%) and fc (120 mg/kg bw/d: 15-19%) during the dosing period.	
16	Post implantation loss	Rabbit	13 (day 6-18)	Oral	10 mg/kg bw/day	**Increase**	At 10 mg/kg bw/day ↑post-implantation loss*, intra-uterine deaths, number of dead implants, attaining significance* (or more meaningful ↑) at ≥30 mg/kg bw/day. Maternal toxicity (↓body weight) at ≥30 mg/kg bw/day.	
17	Post implantation loss	Rabbit	13 (GD 8-20)	Oral	60 mg/kg bw/day	**Increase**	At top-dose 60 mg/kg bw/day: ↓foetal weight, ↑post-implantation losses, intra-uterine deaths (↓live foetuses *in utero* at study termination). At ≥20 mg/kg bw/day maternal animals showed general toxicity (↓body weight and body weight gain).	
37	Post implantation loss	Rabbit	13 (GD 8-20)	Oral	40 mg/kg bw/day	**Increase**	Significant ↑PIL at 40 mg/kg bw/d (22%) and 60 mg/kg bw/day (41%) *vs* controls (~6%) with dose-response but in the presence of significant ↓maternal body weight gain, ↓fc and ↑clinical signs from 20 mg/kg bw/day onwards over the study period.	
13	Pre implantation loss	Rat	GD 7-16	Oral	> 20 mg/kg bw/day	No effect	Not relevant	Not relevant
15	Pre implantation loss	Rat	10 (GD 6-15)	Oral	10 mg/kg bw/day	No effect	Not relevant	

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
16	Pre implantation loss	Rabbit	13 (day 6-18)	Oral	>100 mg/kg bw/day	No effect	Not relevant	
17	Pre implantation loss	Rabbit	13 (GD 8-20)	Oral	>60 mg/kg bw/day	No effect	Not relevant	
37	Pre implantation loss	Rabbit	13 (GD 8-20)	Oral	>60 mg/kg bw/day	No effect	Notifier reported a dose-related n.s.s. ↑pre-implantation loss Notifier reported ↓maternal body weight, ↓fc, and ↓faeces at this dose level, and concluded that the increase in preimplantation loss is not considered an EAS-related effect. RMS considers that in a developmental toxicity assay where treatment begins after implantation, pre-implantation loss is not considered treatment-related.	
13	# anomalies[$]	Rat	GD 7-16	Oral	80 mg/kg bw/day	**Increase**	Top-dose: 1 meningocoele	The observed potential effects of metam sodium on foetal morphological development are considered as the result of retarded development due to general fetotoxicity resulting from observed toxicity in maternal animals. **In conclusion, no adverse EAS-related effect on the incidence of anomalies was observed.**
14	# anomalies[$]	Rat	10 (GD 7-16)	Oral	5 mg/kg bw/day	**Increase**	≥5 mg/kg bw/day: ↑unossified odontoid, cervical vertebrae and calcaneum (variants); a minor defect in a cervical vertebrae center. ≥20 mg/kg bw/day: ↓foetal weight, ↑several vertebral column ossification delays. **Top-dose (60 mg/kg bw/day):** ↑PIL, intra-uterine deaths, 1 unossified cervical arches, 2/2 microphthalmia, 1 anophthalmia, 1 short upper jaw/cleft lip, 3/3 internal hydrocephaly, 1 cerebral meningocoele, ↑manus/pes scores (↓ossification). The top-dose malformations and slighter effects at lower doses are associated with severe maternal / foetal systemic toxicity. Overt maternal toxicity at 20 mg/kg bw/day and above: ↓body weight (gain), ↓fc, ↑salivation, ↑stain around mouth, ↑urinary incontinence, ↑vaginal bleeding, ↑kidney pelvic dilatation. RMS agrees that the anomaly findings are more likely related to the general poor state of the animals and are not of evident EAS-adversity.	

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
15	# anomalies[$]	Rat	10 (GD 6-15)	Oral	10 mg/kg bw/day	**Increase**	↑dose-related incidence of skeletal variations and retardations (ossification delays). ≥40 mg/kg bw/day: ↓foetal weight gain **Top-dose 120 mg/kg bw/day:** 2/1 meningocoele, 1 bilateral microphthalmia. Disagree with notifier that *«no effect on the number of general, skeletal anomalies was observed»*. It is noted that overt maternotoxicity under the form of ↓body weight (gain) and ↓fc appears at 40 mg/kg bw/day and above. RMS considers that the anomaly findings are more likely related to the general poor state of the animals and are not EAS-related.	
16	# anomalies[$]	Rabbit	13 (day 6-18)	Oral	10 mg/kg bw/day	**Increase**	≥10 mg/kg bw/day: ↑PIL, intra-uterine deaths, number of dead implants, ↑visceral variations at ≥30 mg/kg bw/day: gall bladder agenesis, asymetrical sternebrae. **Top-dose 100 mg/kg bw/day:** ↑truncus arteriosus communis, 1 spina bifida, 1 meningocoele. It is noted that overt maternotoxicity under the form of ↓body weight gain appears at ≥30 mg/kg bw/day. RMS considers that the anomaly findings are more likely related to the general poor state of the animals and are not EAS-related.	
17	# anomalies[$]	Rabbit	13 (GD 8-20)	Oral	20 mg/kg bw/day	**Increase**	≥20 mg/kg bw/day: ↑minor skeletal defects, ↓ossification **Top-dose 60 mg/kg bw/day:** ↓foetal weight, ↑PIL, intra-uterine deaths, 1 cerebral meningocoele, 2/2 cleft palate. It is noted that overt maternotoxicity under the form of ↓body weight gain and ↓fc appears at ≥20 mg/kg bw/day. RMS considers that the anomaly findings are more likely related to the general poor state of the animals and are not EAS-related.. The explanation of the notifier, claiming no dose-response (meningocoele, cleft palate) as only observed at high dose, and attributing the effects *«on the decreased number of viable foetuses»*, suggesting non-relevance of the endpoint is not agreed upon. However, no link with endocrine activity is suggested overall, either.	
18	# anomalies[$]	Rat	11	Oral	>12 mg/kg bw/day	No effect		

ID	Effect target	Spe-cies	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
37	# anomalies[$]	Rabbit	13 (GD 8-20)	Oral	40	**Increase**	≥40 mg/kg bw/day: ↓foetal weight, ↑PIL, intra-uterine deaths. **Top-dose 60 mg/kg bw/day:** 1 cyclopia. It is noted that overt maternotoxicity under the form of ↓faecal output, ↓body weight gain, ↓fc appears at ≥20 mg/kg bw/day, and at top-dose (↑sloughed mucosa glandular stomach). RMS considers that the anomaly finding is are more likely related to the general poor state of the animals and is not EAS-related.	
16	Sex ratio	Rabbit	13 (GD 6-18)	Oral	>100 mg/kg bw/day	No effect		A change in sex ratio was observed in one prenatal developmental toxicity study in rabbits. However, no consistent trend was observed within this study and no effect on sex ratio was observed in another study at higher doses. Therefore, **no EAS-related adversity with respect to sex ratio was observed.**
17	Sex ratio	Rabbit	13 (GD 8-20)	Oral	60 mg/kg bw/day	**Change**	20 mg/kg bw/d: highly sign. ↑♂ foetuses (possibly explained by low control value). **Top-dose 60 mg/kg bw**: sign. ↓♂ foetuses; litters containing ≤5 foetuses did not include any ♂. It was noted that the top-dose of 60 mg/kg bw/d was considerably maternotoxic given the ↑embryonic death (9 total resorptions) and ↑post implantation deaths in the 9 dams with viable litters.	
18	Time to mating	Rat	11	Oral	>12 mg/kg bw/day	No effect		**No EAS-related adversity was observed with regards to fertility (time to mating).**

Remark: numerical indications of structural defects expressed on a foetal (1st number)/litter (2nd number) base.
[$]: external, visceral, skeletal anomalies included

Assessment of the integrated line of evidence of fertility/developmental «sensitive to but not diagnostic of EATS» (*in-vivo*) parameters: further discussion and conclusions

Fertility/developmental parameters including time to meeting, gestation length, litter size or viability, number of live births, sex ratio were not affected by treatment with metam.

RMS considers that changes of other fertility/developmental parameters, like litter/pup weight, numbers of embryonic/foetal deaths and viable foetuses, number of implantations, post-implantation loss or presence of anomalies, are more likely related to the general poor state of the animals or of the dams, and considering the toxicity of metam, the findings are not of evident EAS-adversity. In conclusion, in RMS opinion, there is no indication for endocrine-related adversity based on "sensitive to, but not diagnostic of EATS" parameters.

2.2.2.2 MITC

Lines of evidence for the EAS-modalities of MITC are included in **table 2.2.2.2-1 through 2.2.2.2-5 .**
RMS also revised and expanded the *in-vitro* data extracted from the EDSP database of the US EPA.
Details of the evaluation of the EDSP *in-vitro* studies (US EPA) are in Vol.3 B.6.8.3.
See tables 2.2.2.2-1 through 2.2.2.2-5 below:

Table **2.2.2.2-1:** MITC Lines of evidence: EAS modalities, *in-silico* and *in-vitro* studies

ID	Effect classification	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence
n.a.	In silico prediction	Androgen receptor	n.a.	n.a.	n.a.	n.a.	No effect	Molecular docking predictions on binding affinities show low potential. Due to the quality of COMPARA consensus predictions in combination with other models (predicting no androgenic activity), the assessment of androgenic activity based on the available models is considered reliable.	The available in silico data provides supporting evidence that MITC has no androgenic activity.
22	In vitro mechanistic	Androgen receptor	n.a.	n.a.	n.a.	-	No effect	The ToxCast AR prediction model value is 0 for agonistic and antagonistic activity. In the cases where equivocal responses were observed for metam, the similar in-vitro assays came out negative for MITC.	Based on the results of the ToxCast AR model no indication for androgenic activity was observed.
n.a.	In silico prediction	Oestrogen receptor	n.a.	n.a.	n.a.	n.a.	No effect	Molecular docking predictions on binding affinities show low potential. Considering the results of the predictions as provided above in a weight of evidence the overall concern with regard to potential estrogenic activity is considered low. The overall weight of the CERAPP predictions is considered highest due a) the consensus approach and b) the extensive training and validation set derived within the CERAPP project.	The available in silico data provides supporting evidence that MITC has no oestrogenic activity.
-*	In vitro mechanistic	Oestrogen receptor (E-modality)	Human breast cell line, VM7	22h	Uptake from the medium (in vitro)	-	No effect	RMS : TOX21_ERa_LUC_VM7_Agonist High-variable concentration response, with only ↑top-concentration (100 µM) around cut-off; it is of note that a.s. metam displays the same behaviour in this assay.	Based on the results of the ToxCast ER model no indication for oestrogenic activity was observed.
21	In vitro mechanistic	Oestrogen receptor	n.a.	n.a.	n.a.	-	No effect	The ToxCast ER prediction model value is 0 for agonistic and antagonistic activity.	
n.a.	In silico prediction	Steroidogenesis	n.a.	n.a.	n.a.	n.a.	No effect	Only one model is available for steroid receptors: glucocorticoid receptor (GR). For both compounds, steroid activity is evaluated by means of the molecular docking method (COSMOS). The results indicate low probability of binding.	The available in silico data provides supporting evidence that MITC has no steroidal activity.

ID	Effect classification	Effect target	Cell system	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence
23	In vitro mechanistic	CYP19	Human, cell-free	0.5h	Uptake from the medium (in vitro)	3.63µM	Equivocal (notifier: *«no effect»)*	NVS_ADME_hCYP19A1: biochemical assay to monitor human CYP19A1 activity inhibition. Flags: multiple points above baseline, but response below cut-off; relevance of the slightly increased activity unexplained, since a signal direction type other than loss is unexpected in case of aromatase inhibition.	The available in vitro mechanistic data based on results of the CompTox Database are negative. The occasional single slight upregulation of CYP19A1 is of uncertain significance in the absence of other aromatase assay findings, and as aromatase is a key enzyme of oestradiol production, the finding would be reflected at that level too, which is not the case. Thus, there is no indication for endocrine activity (S modality) based on in vitro mechanistic data.
24	In vitro mechanistic	CYP19	Human breast cell line, MCF-7	24h	Uptake from the medium (in vitro)	-	No effect	TOX21_Aromatase_Inhibition	
25	In vitro mechanistic	CYP19	Human breast cell line, MCF-7	24h	Uptake from the medium (in vitro)	-	No effect	TOX21_Aromatase_Inhibition_viability	

Assessment of each line of evidence and integrated lines of evidence EAS modality (in-silico and in-vitro): further discussion and conclusions

The available *in silico* prediction and *in-vitro* data provides supporting evidence that MITC has no androgenic, oestrogenic or stereoidogenesis activity.

Table 2.2.2.2-2: MITC Lines of evidence: EAS mediated, *in-vivo* studies, males

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
3	Epididymis weight	Rat	28d	Inhalation	>120 µg/L	No effect		No coherent adverse effect on epididymis weight was observed. Observed epididymis weight changes did not correspond to histopathological changes. In conclusion, EAS-mediated adversity with respect to effects on epididymis was not observed.
4	Epididymis weight	Mouse	28d	Inhalation	60 µg/L	**Increase**	↑relative weight associated with ↓body weight	
10a	Epididymis weight	Rat	♂: 28d ♀: 58-67d	Inhalation	20 ppm	**Decrease**	↓relative (to brain weight) and ↓absolute R epididymis weight associated with ↓final body weight	
12	Epididymis weight	Rat	F_0-F_1 adults	Inhalation	20 ppm	**Increase**	F_0: n.s.s. ↓absolute epididymis weight, ↑relative epididymis weight associated with ↓body weight F_1: sign. ↑relative epididymis weight associated with ↓body weight Effects on organ weight are associated to ↓final body weight in both F_0 and F_1. Moreover, no histopathological changes were observed in epididymides and thus weight changes are not considered adverse effects.	
15	Epididymis weight	Mouse	78wk	Inhalation	1 ppm	**Increase**	Dose-dependent sign. ↑relative epididymides weight. However, no histopathological changes were observed in epididymides at the top-dose, and thus weight changes are not considered adverse effects.	
16	Epididymis weight	Rat	52-104wk	Inhalation	20 ppm	**Decrease**	↓absolute and relative (to brain) epididymis weight (significant wk 52, not sign. wk 104) as well as a significant ↑relative organ to body weight (wk 104). As no consistent effect was observed, the weight changes were not considered toxicologically relevant.	
19	Epididymis weight	Rat	13wk	Inhalation	>15 ppm	No effect		
1	Epididymis histopathology	Dog	90d	Oral	>2 mg/kg bw/day	No effect		
12	Epididymis histopathology	Rat	F_0 & F_1 adults	Inhalation	> 20 ppm	No effect		
15	Epididymis histopathology	Mouse	78wk	Inhalation	>15 ppm	No effect		
16	Epididymis histopathology	Rat	52-104wk	Inhalation	> 20 ppm	No effect		
17	Epididymis histopathology	Mouse	26-52-104wk	Oral	> 200 ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
18	Epididymis histopathology	Rat	53-104wk	Oral	> 50 ppm	No effect		
19	Epididymis histopathology	Rat	13wk	Inhalation	>15 ppm	No effect		
20	Epididymis histopathology	Mouse	13wk	Inhalation	>20 ppm	No effect		
1	Testis weight	Dog	90d	Oral	0.4 mg/kg bw/day	**Decrease**	sign. ↓relative testis weight not associated with ↓final body weight. The change is relevant compared to concurrent but not to HCD. As no histopathological change was observed, the ↓ in testis weight is not considered adverse.	No coherent adverse effect on testis weight was observed. Observed testis weight changes did not correspond to histopathological changes. In conclusion, EAS-mediated adversity with respect to effects on testis was not observed.
2	Testis weight	Rat	28d	Inhalation	>100 µg/L	No effect		
3	Testis weight	Rat	28d	Inhalation	>120 µg/L	No effect		
4	Testis weight	Mouse	28d	Inhalation	60 µg/L	**Increase**	↑relative testis weight associated with ↓final body weight. Due to this and lacking histopathological changes in the chronic study in mouse, the increased relative testis weight is not considered adverse.	
10a	Testis weight	Rat	♂:28d, ♀:58-67d	Inhalation	>20 ppm	No effect		
11	Testis weight	Rat	16-19wk	Oral	>50 ppm	No effect		
12	Testis weight	Rat	F_0-F_1 adults	Inhalation	20 ppm	**Increase**	sign. F_0-F_1: ↑relative testis weight associated with ↓body weight (analysed together with epididymides and *vas deferens*). This effect is not considered relevant as no histopathological changes were observed.	
15	Testis weight	Mouse	78wk	Inhalation	5 ppm	**Increase**	significantly ↑relative testes weights [relative to body weight (5, 15 ppm) and brain weight (15 ppm)] only associated to ↑final b.w. at the top-dose, of 15 ppm but not at the lower dose.	
16	Testis weight	Rat	52-104wk	Inhalation	20 ppm	**Decrease**	sign. ↓relative (to body weight, wk 52, 104) and absolute testis weights (wk 104) associated with ↓body weight. This effect is not considered relevant as no histopathological changes were observed.	
17	Testis weight	Mouse	26-52-104wk	Oral	>200 ppm	No effect		
18	Testis weight	Rat	53-104wk	Oral	>50 ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
19	Testis weight	Rat	13wk	Inhalation	>20 ppm	No effect		
20	Testis weight	Mouse	13wk	Inhalation	>20 ppm	No effect		
1	Testis histopathology	Dog	90d	Oral	>2	No effect		
11	Testis histopathology	Rat	16-19wk	Oral	> 50	No effect		
12	Testis histopathology	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
15	Testis histopathology	Mouse	78wk	Inhalation	>15 ppm	No effect		
16	Testis histopathology	Rat	52-104wk	Inhalation	> 20 ppm	No effect		
17	Testis histopathology	Mouse	26-52-104wk	Oral	> 200 ppm	No effect		
18	Testis histopathology	Rat	26-52-104wk	Oral	>50 ppm	No effect		
19	Testis histopathology	Rat	13wk	Inhalation	>15 ppm	No effect		
20	Testis histopathology	Mouse	13wk	Inhalation	>20 ppm	No effect		
11	Seminal vesicles weight	Rat	16-19wk	Oral	>50 ppm	No effect		No adverse effects on seminal vesicles histopathology were observed. In conclusion, EAS-mediated adversity with respect to effects on seminal vesicles was not observed.
17	Seminal vesicles weight	Mouse	26-52-104wk	Oral	> 200	No effect		
12	Seminal vesicles histopathology	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
15	Seminal vesicles histopathology	Mouse	78wk	Inhalation	>15 ppm	No effect		
16	Seminal vesicles histopathology	Rat	52-104wk	Inhalation	> 20 ppm	No effect		
17	Seminal vesicles histopathology	Mouse	26-52-104wk	Oral	> 200 ppm	No effect		
18	Seminal vesicles histopathology	Rat	53-104wk	Oral	>50 ppm	No effect		
1	Prostate weight	Dog	90d	Oral	>2 mg/kg bw/day	No effect		No adverse effect on prostate was observed. Prostate weight changes (observed in 2 rat studies) may be attributed to general systemic toxicity observed in these studies at the corresponding dose level. No histopathological changes were observed. In conclusion, EAS-mediated adversity
11	Prostate weight	Rat	F_1-F_2 litter	Oral	>50 ppm	No effect		
12	Prostate weight	Rat	F_0-F_1 adults	Inhalation	20 ppm	**Increase**	F_0: sign. ↑relative prostate weight associated with ↓body weight, F_1: no effect. This effect is therefore not considered relevant, and in addition no histopathological changes were observed in the prostate, in neither F_0 nor F_1.	
15	Prostate weight	Mouse	78wk	Inhalation	>15 ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
16	Prostate weight	Rat	52-104wk	Inhalation	20 ppm	**Decrease**	↓absolute and relative (to brain) prostate weight (sign. after 104 wks exposure only) associated with ↓body weight, Moreover, no histopathological changes were observed.	with respect to effects on prostate was not observed.
17	Prostate weight	Mouse	26-52-104wk	Oral	>200 ppm	No effect		
1	Prostate histopathology*	Dog	90d	Oral	>2 mg/kg bw/day	No effect		
11	Prostate histopathology*	Rat	16-19wk	Oral	>50 ppm	No effect		
12	Prostate histopathology*	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
15	Prostate histopathology*	Mouse	78wk	Inhalation	>15 ppm	No effect		
16	Prostate histopathology*	Rat	52-104wk	Inhalation	>20 ppm	No effect		
17	Prostate histopathology*	Mouse	26-52-104wk	Oral	>200 ppm	No effect		
18	Prostate histopathology*	Rat	53-104wk	Oral	>50 ppm	No effect		
15	Accessory sex organs histopathology	Mouse	78wk	Inhalation	>15 ppm	No effect		No adverse effects on accessory sex organs and coagulating gland histopathology was observed. In conclusion, EAS-mediated adversity with respect to effects on accessory sex organs as well as coagulating gland was not observed.
16	Accessory sex organs histopathology	Rat	52-104wk	Inhalation	>20 ppm	No effect		
12	Coagulating gland histopathology	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
12	Sperm numbers	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		No adverse effects on sperm parameters were observed in a 2 Generation study. In conclusion, EAS-mediated adversity with respect to effects on sperm parameters was not observed.
12	Sperm morphology	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
12	Sperm motility	Rat	F_0-F_1 adults	Inhalation	>20 ppm	No effect		
12	Age at balanopreputial separation	Rat	litter F1 & F2	Inhalation	> 20ppm	No effect	Balanopreputial separation was comparable across all groups (0 ppm: 49.5d, 1 ppm: 51.3d, 5 ppm: 48.3d, 20 ppm: 48.1d) and the HCD of the laboratory (43.0-49.0 d).	No EAS-mediated adversity with respect to balanopreputial separation was observed.
12	Ano-Genital distance	Rat	litter F1 & F2	Inhalation	> 20ppm	No effect		No EAS-mediated adversity with respect to effects on anogenital distance was observed.

*: including seminal vesicles and coagulating glands

Assessment on the integrated line of evidence EAS modality, *in-vivo* studies, males:

In conclusion, there is no indication for endocrine-related adversity based on "EATS-mediated" parameters with regards to EAS modalities.

Table **2.2.2.2-3**: MITC Lines of evidence: EAS mediated, *in-vivo* studies, females

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
1	Ovary weight	Dog	90d	Oral	>2 mg/kg bw/day	No effect		No coherent effect on ovaries was observed. Observed absolute ovary weight changes (↓of absolute and ↑of relative organ weight) may be attributed to general systemic toxicity observed in these studies at the corresponding dose level. Histopathological changes were observed in 1 chronic study in mouse only, which were not statistically significant. In conclusion, EAS-mediated adversity with respect to effects on ovaries was not observed.
3	Ovary weight	Rat	28d	Inhalation	>120 µg/L	No effect		
4	Ovary weight	Mouse	28d	Inhalation	>120 µg/L	No effect		
10a	Ovary weight	Rat	♂: 28d ♀: 58-67d	Inhalation	20ppm	**Decrease**	↓absolute ovaries/oviduct weight. At this dose level also signs of general systemic toxicity were observed (*i.e.* ↓body weight gain and ↓food consumption) and thus, ↓organ weight is not considered EAS-mediated.	
11	Ovary weight	Rat	16-19wk	Oral	>50ppm	No effect		
11	Ovary weight	Rat	litter F1	Oral	10ppm	**Increase**	Sign. ↑relative ovary weight (of 10 and 50 ppm group). It is considered that these results are most probably a spurious effect, exacerbated by the technical difficulty in removing and weighing organs of this size.	
11	Ovary weight	Rat	litter F2	Oral	>50ppm	No effect		
12	Ovary weight	Rat	F0 & F1 adults	Inhalation	20ppm	**Decrease**	F1 adults: sign. ↓ovary weight (absolute only). This effect is not considered relevant as no histopathological changes were observed.	
12	Ovary weight	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect	F2 adults: No effect on ovary weight (absolute and relative) was observed.	
15	Ovary weight	Mouse	78wk	Inhalation	>15ppm	No effect	n.s.s. ↓ovary weight (absolute, relative) at 5ppm and 15ppm, however lacking dose-dependency (changes equal at these 2 doses), and in the absence of histopathological findings not considered toxicologically relevant.	
16	Ovary weight	Rat	52-104wk	Inhalation	> 20ppm	No effect		
17	Ovary weight	Mouse	26-52-106wk	Oral	> 200ppm	No effect		
18	Ovary weight	Rat	53-104wk	Oral	> 50ppm	No effect		
19	Ovary weight	Rat	13wk	Inhalation	> 15ppm	No effect		
20	Ovary weight	Mouse	13wk	Inhalation	> 20ppm	No effect		

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
1	Ovary histopathology	Dog	90d	Oral	>2 mg/kg bw/day	No effect		
11	Ovary histopathology	Rat	16-19wk	Oral	> 50ppm	No effect		
12	Ovary histopathology	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect		
15	Ovary histopathology	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Ovary histopathology	Rat	52-104wk	Inhalation	> 20ppm	No effect		
17	Ovary histopathology	Mouse	26-52-106wk	Oral	200ppm	**Change**	Week 52: No ↑in absent *corpus luteum* observed. Week 106: non-sign.↑ in ovary cysts. No ↑ in absent *corpus luteum* observed. At top-dose of 200 ppm there is general toxicity such as ↓body weight and ↓fc at ≥ 80 ppm), thus the n.s.s. ↑ovary cysts is unlikely attributed to EAS-mediated adversity. No such finding in the inhalation study.	
18	Ovary histopathology	Rat	53-104wk	Oral	50ppm	**Change**	Week 53: single ♀ observed with ovarian bursal cyst (1/5) Week 104: non-sign.↑ in ovary cysts (22% at top-dose *vs.* 11% in ctr). No data on CL counts. At top-dose of 50 ppm there is general toxicity such as ↓body weight), thus the n.s.s. ↑ovary cysts is unlikely attributed to EAS-mediated adversity. No such finding in the inhalation study.	
19	Ovary histopathology	Rat	13wk	Inhalation	> 15ppm	No effect		
20	Ovary histopathology	Mouse	13wk	Inhalation	> 20ppm	No effect		
19	Oviduct histopathology	Rat	13wk	Inhalation	> 15ppm	No effect		
20	Oviduct histopathology	Mouse	13wk	Inhalation	> 20ppm	No effect		
1	Uterus weight*	Dog	90d	Oral	>2mg/kg bw/day	No effect		No coherent effect on uterus was observed. Observed uterus weight changes were not corroborated with histopathological changes (RMS: in case of weight increases, this is not necessarily associated *e.g.* in cases of hydrometra, but the latter has not been observed and
5	Uterus weight*	Rat	9d (from postcoital Day 6-15)	Oral	> 30 mg/kg bw/day	No effect		
6	Uterus weight*	Rabbit	12d (from postcoital Day 6-18)	Oral	> 10 mg/kg bw/day	No effect		
7	Uterus weight*	Rabbit	21d (GD 7-28)	Inhalation	> 15ppm	No effect		

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
8	Uterus weight*	Rabbit	21d (GD 7-28)	Inhalation	> 12ppm	No effect		both ↑ and ↓ weight has been measured). No effects on cervix were observed. In conclusion, EAS-mediated adversity with respect to effects on uterus and cervix was not observed.
11	Uterus weight*	Rat	16-19wk	Oral	> 50ppm	No effect		
11	Uterus weight*	Rat	litter F1 & F2	Oral	> 50ppm	No effect		
12	Uterus weight*	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect	F0 adults: No effect on uterus weight (absolute and relative) was observed.	
12	Uterus weight*	Rat	F0 & F1 adults	Inhalation	20ppm	**Increase**	F1 adults: ↑sign. uterus weight (relative only). This effect is not considered relevant as no histopathological changes were observed.	
13	Uterus weight*	Rat	15d (GD 6-20)	Inhalation	20ppm	**Decrease**	↓gravid uterus weights correlating with ↓foetal weights and general maternal toxicity (↓body weight and ↓fc) and thus, this effect is not considered EAS-related.	
14	Uterus weight*	Rat	16d (GD 6-20)	Inhalation	> 12ppm	No effect		
15	Uterus weight*	Mouse	78wk	Inhalation	15ppm	**Decrease**	sign. ↓uterus weights (absolute and relative to body and brain weight) at top-dose where general systemic toxicity was observed (↓body weight, ↓fc). As no histopathological changes were observed, the ↓uterus weight is not considered EAS-related.	
16	Uterus weight*	Rat	52-104wk	Inhalation	> 20ppm	No effect		
19	Uterus weight*	Rat	13wk	Inhalation	> 15ppm	No effect		
20	Uterus weight*	Mouse	13wk	Inhalation	> 20ppm	No effect		
1	Uterus histopathology*	Dog	90d	Oral	>2mg/kg bw/day	No effect		
11	Uterus histopathology*	Rat	16-19wk	Oral	> 50ppm	No effect		
12	Uterus histopathology*	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect	No MITC-related effects were observed on uterus and cervix.	
15	Uterus histopathology*	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Uterus histopathology*	Rat	52-104wk	Inhalation	> 20ppm	No effect		
17	Uterus histopathology*	Mouse	26-52-106wk	Oral	> 200ppm	No effect		
18	Uterus histopathology*	Rat	53-104wk	Oral	> 50ppm	No effect		

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
19	Uterus histopathology*	Rat	13wk	Inhalation	> 15ppm	No effect		
15	Cervix histopathology	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Cervix histopathology	Rat	52-104wk	Inhalation	> 20ppm	No effect		
1	Vagina histopathology	Dog	90d	Oral	>2mg/kg bw/day	No effect		
15	Vagina histopathology	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Vagina histopathology	Rat	52-104wk	Inhalation	> 20ppm	No effect		
1	Mammary gland histopathology	Dog	90d	Oral	>2mg/kg bw/day	No effect		No relevant EAS-mediated adversity with respect to effects on mammary gland was observed.
12	Mammary gland histopathology	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect		
15	Mammary gland histopathology	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Mammary gland histopathology	Rat	52-104wk	Inhalation	> 20ppm	No effect		
18	Mammary gland histopathology	Rat	53-104wk	Oral	> 50ppm	No effect		
18	Mammary gland histopathology	Rat	52-104wk	Oral	50ppm	**Change**	At termination in top-dose ♀, n.s.s. slight ↑multiple benign mammary tumours compared to than in controls. In the absence of single benign and malignant mammary tumours in this assay, and in the absence of mammary tumours in the more recent inhalation long-term-study, **RMS** considers the elevation non relevant.	
12	Oestrus cyclicity	Rat	F0 & F1 adults	Inhalation	20ppm	**Increase**	sign. ↑mean oestrous cycle length at top-dose in both F0 (4.5 days) and F1 (4.9d) *vs.* concurrent control group (4.1 days F0 & F1). The oestrus cycle length of F0 and F1 females at 20 ppm was within the range of HCD of the laboratory (4.0 to 5.8 days) and longer oestrus cycles in F0 and F1 were attributable to 2 (F0) or 1 (F1) animals only. Therefore, the effect was not considered toxicologically relevant by the notifier, but **RMS** considers that this weak modification occurring in both generations, and	According to the notifier, no relevant EAS-mediated adversity with respect to effects on oestrus cycle was observed. However, top-dose effects were not considered non-relevant by the RMS. The cause of this slight increase was not clear, but any interference with high systemic toxicity seems plausible, and RMS

Id	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
							displaying some dose-responsiveness cannot be ignored.	does not think that the finding points to an EAS-mediated mechanism, also because there was no overt reprotoxicity in 2 multigeneration studies conducted with MITC.
12	Age at Vaginal opening	Rat	litter F1 & F2	Inhalation	20ppm	**Increase**	sign. ↑vaginal patency delayed (control: PND 34.4; 20 ppm: PND 36.5). While the values of treatment groups are within the HCD and thus, not considered toxicologically relevant by the notifier, **RMS** considers it potentially relevant.	According to the notifier, no relevant EAS-mediated adversity with respect to effects on vaginal patency was observed. However, top-dose effects were not considered non-relevant by the RMS. The cause of this slight increase was not clear, but any interference with high systemic toxicity seems plausible, and RMS does not think that the finding points to an EAS-mediated mechanism, also because there was no overt reprotoxicity in 2 multigeneration studies conducted with MITC.
12	Ano-Genital distance	Rat	litter F1 & F2	Inhalation	> 20ppm	No effect		No EAS-mediated adversity with respect to effects on anogenital distance was observed.

*with cervix

Assessment on the integrated line of evidence EAS modality, *in-vivo* studies, females:
In conclusion, there is no indication for endocrine-related adversity based on "EATS-mediated" parameters with regards to EAS modalities.

Table **2.2.2.2-4:** MITC Lines of evidence: sensitive to, but not diagnostic of EATS, *in-vivo* studies

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
1	Pituitary weight	Dog	90d	Oral	>2mg/kg bw/day	No effect		Relevant adverse effects on pituitary were not observed. Increases in pituitary weight did not correspond to histopathological changes. Moreover, general toxicity was observed at the same exposure concentration levels (significant reductions in bw). In conclusion, adversity sensitive to but not diagnostic of EATS modality was not observed for MITC based on the absence of relevant pituitary weight or histopathological changes.
10a	Pituitary weight	Rat	♂: 28d ♀: 58-65d	Inhalation	> 20ppm	No effect		
11	Pituitary weight	Rat (F0, adult)	16-19wk	Oral	50ppm	**Increase**	sign. ↑absolute and relative pituitary weight (♀ only). Without meaningful histopathological correlate, this effect is not considered EATS related.	
11	Pituitary weight	Rat (F1, adult)	16-19wk	Oral	> 50ppm	No effect	No histopathology.	
11	Pituitary weight	Rat	litter F1 & F2	Oral	> 50ppm	No effect		
12	Pituitary weight	Rat	F0 & F1 adults	Inhalation	20ppm	**Increase**	sign. ↑relative pituitary weight (♂,♀), associated with sign. ↓body weight and ↓fc. This effect is not considered relevant in the absence of histopathological changes.	
15	Pituitary weight	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Pituitary weight	Rat	52-104wk	Inhalation	> 20ppm	No effect	n.s.s. ↓abs + rel. pituitary weight at top-dose and at wk104 not considered toxicologically significant.	
17	Pituitary weight	Mouse	26-52-106wk	Oral	80 ppm	**Increase**	Wk 26: sign. ↑relative pituitary weight at 200 ppm (♀ only). Wk 52: sign. ↑absolute + relative pituitary weight at 80 ppm (♀ only) and at 200 ppm (♂+♀), the latter without dose-dependency. Wk 106: sign. ↑relative pituitary weight at 200 ppm (♀ only). Slight ↓body weight and ↓fc as a sign of general systemic toxicity. Without histopathological correlate, this effect is not considered EATS related.	
18	Pituitary weight	Rat	53-104wk	Oral	> 50ppm	No effect		
1	Pituitary histopathology	Dog	90d	Oral	>2mg/kg bw/day	No effect		
11	Pituitary histopathology	Rat (F0, adult)	16-19wk	Oral	> 50ppm	No effect	**RMS**: a single pituitary cyst was observed in the top-dose animals: 1/10 examined ♂	

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
							and 1/13 examined ♀, *vs.* no observed lesions in the concomitant controls.	
12	Pituitary histopathology	Rat	F0 & F1 adults	Inhalation	> 20ppm	No effect		
15	Pituitary histopathology	Mouse	78wk	Inhalation	> 15ppm	No effect		
16	Pituitary histopathology	Rat	52-104wk	Inhalation	20ppm	**Change**	**RMS:** n.s.s. ↑of both hyperplasia (minimal/mild) of the *pars distalis* and of pituitary cysts (in the ♂, and more pronounced in the ♀), however in the absence of ↑pituitary weight. Not observed in the LT oral rat assay. No ↑pituitary tumours in any rodent assay, and no evidence of any effect on the T- or E-modality. Overall, the effect, if of any relevance, is unlikely to be considered EATS related.	
17	Pituitary histopathology	Mouse	26-52-106wk	Oral	>200ppm	No effect		
18	Pituitary histopathology	Rat	53-104wk	Oral	>50ppm	No effect		
1	Adrenals weight	Dog	90d	Oral	>2mg/kg bw/day	No effect		Changes in adrenal weight were observed in rat and mouse. However, these changes were not consistent in direction as ↑ and ↓of adrenal weight were observed. Moreover, corresponding histopathological effects were not always observed, except for some cases where weight ↑ correlated with incidences of adrenal angiectasis. Adrenal weight changes were observed at systemic toxic doses resulting in *e.g.* ↓body weight (gain), and are likely explained to stress-related alterations. In conclusion, adversity "sensitive to but not diagnostic of" T modality was observed for MITC based on limited
2	Adrenals weight	Rat	28d	Inhalation	>100µg/L	No effect		
3	Adrenals weight	Rat	28d	Inhalation	>120µg/L	No effect		
4	Adrenals weight	Mouse	28d	Inhalation	60µg/L	**Decrease**	sign. ↓relative adrenal weight. While organ weight was within HCD and the effect is not considered treatment-related.	
11	Adrenals weight	Rat	16-19d	Oral	>50ppm	No effect		
11	Adrenals weight	Rat	litter F1 & F2	Oral	>50ppm	No effect		
12	Adrenals weight	Rat	F0 & F1 adults	Inhalation	20ppm	**Increase**	sign. ↑ mean absolute (♀ F0) and relative (♂ / ♀ F0 & F1) adrenal glands weight, associated with general systemic toxicity (*i.e.* ↓body weight and ↓fc) at top-dose. This effect is not considered EAS related.	

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
15	Adrenals weight	Mouse	78wk	Inhalation	15ppm	**Decrease**	sign. ↓(absolute and relative to brain weight) adrenal glands weight in ♀ only.	evidence of adrenal weight changes with or without histopathological changes. However, the findings may be stress-related due to high systemic toxicity, and not *per se* EAS-related.
16	Adrenals weight	Rat	52-104wk	Inhalation	20ppm	**Increase**	sign. ↑adrenal gland weights (♀: week 52, 104) in line with ↑incidences of angiectasis.	
17	Adrenals weight	Mouse	26-52-106wk	Oral	80ppm	**Increase**	Week 106: sign. ↑adrenal gland weights (absolute and relative) of the adrenal gland in ♀ (right: 200 ppm, left: 80 ppm, poor dose-dependency). No effect in ♂ was observed.	
18	Adrenals weight	Rat	53-104wk	Oral	>50ppm	No effect		
19	Adrenals weight	Rat	13wk	Inhalation	5ppm	**Change**	sign. ↑(only relative to body weight) adrenal glands weight in ♀ only. No dose-dependency in the 2 highest doses (both +14%). No histopathological correlate, either.	
20	Adrenals weight	Mouse	13wk	Inhalation	>20ppm	No effect		
20	Adrenals weight	Mouse	13wk	Inhalation	>20ppm	No effect		
1	Adrenals histopathology	Dog	90d	Oral	>2mg/kg bw/day	No effect		
2	Adrenals histopathology	Rat	28d	Inhalation	>100µg/L	No effect		
12	Adrenals histopathology	Rat	F0 & F1 adults	Inhalation	20ppm	**Change**	F0 ♀: Cytoplasmic vacuolation 2 animals which is considered a background finding that is commonly observed in adult rats and stress-related. RMS: no such finding in F1 ♀.	
15	Adrenals histopathology	Mouse	78wk	Inhalation	>15ppm	No effect		
16	Adrenals histopathology	Rat	52-104wk	Inhalation	20ppm	**Change**	Slightly ↑incidences of adrenal angiectasis (weeks 52, 104). At wk 104, top-dose: both in ♂ and ♀ (much more pronounced in ♀). While it is a common age-related finding and not considered relevant by the notifier, **RMS** considers the effects stress- and thus treatment-related.	
17	Adrenals histopathology	Mouse	26-52-106wk	Oral	>200ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
18	Adrenals histopathology	Rat	53-104wk	Oral	>50ppm	No effect		
19	Adrenals histopathology	Rat	13wk	Inhalation	>15ppm	No effect		
20	Adrenals histopathology	Mouse	13wk	Inhalation	>20ppm	No effect		
5	Fertility (mammals)	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect	Conception rate was not affected.	No EAS-related adversity was observed with regards to reproductive/fertility parameters.
12	Fertility (mammals)	Rat	F0 & F1 adults	Inhalation	>20ppm	No effect	Mating, male/female fertility, copulation, conception indices not affected.	
10a	Fertility (mammals)	Rat	♂: 28 ♀: 58-65	Inhalation	>20ppm	No effect	Mating, fertility, conception, copulation indices not affected.	
11	Fertility (mammals)	Rat	16-19wk	Oral	>50ppm	No effect	Mating, fertility and fecundity index were not affected.	
10a	Gestation length	Rat	♂: 28 ♀: 58-65	Inhalation	>20ppm	No effect		No EAS-related adversity was observed with regards to gestation parameters and delivery.
11	Gestation length	Rat	16-19wk	Oral	> 50	No effect		
11	Gestation length	Rat	16-19wk	Oral	> 50	No effect		
12	Gestation length	Rat	F0 & F1 adults	Inhalation	20ppm	Increase	F0: sign. ↑ in gestation length (22.0d) when compared to the F0 control group (21.6 days). Since the noted gestation length of 22.0 days fell within the laboratory HCD range (21.5-22.3 days), the difference was not considered treatment-related by the notifier, and thus, not toxicologically relevant. F1: n.s.s.↑ in gestation length (22.0d) when compared to the F1 control group (21.7 days). RMS: This marginal modification may be treatment-related, but the magnitude is low and not confirmed in the older generational study. Association with systemic toxicity is probable, and is not EAS-related.	
10a	Dystocia	Rat	♂: 28 ♀: 58-65	Inhalation	>20ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
12	Dystocia	Rat	F0 & F1 adults	Inhalation	>20ppm	No effect		
5	Foetal development	Rat	9d (GD 6-15)	Oral	30mg/kg bw/day	**Increase**	↑n° of runts at top-dose, inducing maternal toxicity only (↓body weight at ≥10 mg/kg bw/d; ↓fc at top-dose). Therefore, ↑n° of runts is not considered specifically EAS-related, but linked to the high general systemic maternotoxicity.	No EAS-related adversity was observed with regards to foetal development.
13	Foetal development	Rat	15d (GD 6-20)	Inhalation	20ppm	**Decrease**	Range-finding study. Sign. ↓foetal body weights (11.4 to 14.3%) correlating with a sign. ↓gravid uterine weight at maternotoxic dose only (↓body weight, ↓fc). Therefore, ↓foetal body weight is not considered specifically EAS-related, but a result of the general systemic maternotoxicity.	
14	Foetal development	Rat	16d (GD 6-20)	Inhalation	12ppm	No effect		
10b	Litter size	Rat	30d	Inhalation	>20ppm	No effect		In 1 /4 studies, the litter size was marginally decreased in the first generation. As this effect was marginal and observed in 1 generation only, with lacking consistency in further studies, no EAS-related adversity was observed with regards to litter size.
11	Litter size	Rat	litter F1	Oral	10ppm	**Decrease**	n.s.s. ↓# pups born/♀ at ≥10 ppm, (F1 generation only).	
11	Litter size	Rat	litter F2	Oral		No effect		
12	Litter size	Rat	litter F1 & F2	Inhalation	>20ppm	No effect		
14	Litter size	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
5	#embryonic or foetal deaths & viable foetuses	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect		In 1 /4 studies, weak effects were observed on viability, however in the presence of strong maternotoxicity. Overall, no EAS-related adversity was observed with regards to the numbers of embryonic or foetal deaths and viable foetuses, litter viability as well as the number of live births.
6	#embryonic or foetal deaths & viable foetuses	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		
7	#embryonic or foetal deaths & viable foetuses	Rabbit	21d (GD 7-28)	Inhalation	15ppm	**Change**	n.s.s. slight ↓foetal viability, ↑early resorption, ↑post implantation in the presence of strong maternotoxicity: sign. ↓body weight gain (-154% d7-29) and ↓fc (-19% d7-29).	

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
14	#embryonic or foetal deaths & viable foetuses	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
12	Litter viability	Rat	litter F1 & F2	Inhalation	>20ppm	No effect		Some weak decreases in pup survival were observed in rat. However, the differences with the control group were not significant, did not occur in a dose-related manner, and were not consistent between generations. Therefore, they are unlikely to be attributed to the treatment and thus also not EAS-related. Therefore, no EAS-related adversity was observed with regards to pup survival.
10b	#live births	Rat	30d	Inhalation	>20ppm	No effect		
10b	Pup survival index	Rat	30d	Inhalation	>20ppm	No effect		
11	Pup survival index	Rat	litter F1	Oral	2ppm	**Decrease**	n.s.s. ↓viability indices in all treatment groups, but without dose-response relationship. Since control pup mortality values were unusually low, the higher incidences of pup deaths in treated groups were considered to be unrelated to treatment.	
11	Pup survival index	Rat	litter F2	Oral	10ppm	**Decrease**	n.s.s. ↓viability index at d21/d1 only ≥10ppm. Effect even weaker than in F1. Overall considered to be unrelated to treatment.	
12	Pup survival index	Rat	litter F1 & F2	Inhalation	1ppm	**Decrease**	F1: n.s.s. ↓postnatal survival PND4-PND 28 in all test groups during PND 7-14 and PND 4 (post-cull) to PND 28 *vs.* control group. These differences could be attributed to 2, 3, and 2 F0 ♀ in the 1, 5, and 20 ppm groups which showed total litter losses. **RMS:** the differences with the control group were not significant, did not occur in a dose-related manner, and were not consistent between generations (no effect in F2); therefore, they are unlikely to be attributed to the treatment and thus also not EAS-related.	
12	Pup survival index	Rat	litter F1 & F2	Inhalation	>20ppm	No effect		
5	Litter/pup weight	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect	No meaningful ↓mean foetal weight (-0.8%) at top-dose; a ↑runt incidence (#foetuses weighing < 75% of the mean foetal weight/litter) was observed, but apparently without influence on the average weight.	No relevant EAS-related adversity was observed with regards to foetal, litter/pup weight.

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
6	Litter/pup weight	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		
7	Litter/pup weight	Rabbit	21d (GD 7-28)	Inhalation	>15ppm	No effect		
8	Litter/pup weight	Rabbit	21d (GD 7-28)	Inhalation	>12ppm	No effect		
11	Litter/pup weight	Rat	litter F1 & F2	Oral	>50ppm	No effect		
12	Litter/pup weight	Rat	litter F1 & F2	Inhalation	>20ppm	No effect		
14	Litter/pup weight	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
6	#implantations, corpora lutea	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		No EAS-related adversity was observed with regards to the number of implantations, corpora lutea.
7	#implantations, corpora lutea	Rabbit	21d (GD 7-28)	Inhalation	>15ppm			
8	#implantations, corpora lutea	Rabbit	21d (GD 7-28)	Inhalation	>12ppm	No effect	range-finding study	
14	#implantations, corpora lutea	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
5	Pre implantation loss	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect	Not relevant	Pre-implantation loss effects are not relevant for assays where the test article is administered after implantation.
6	Pre implantation loss	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect	Not relevant	
7	Pre implantation loss	Rabbit	21d (GD 7-28)	Inhalation	>15ppm	No effect	Not relevant	
8	Pre implantation loss	Rabbit	21d (GD 7-28)	Inhalation	>12ppm	No effect	Not relevant	
14	Pre implantation loss	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect	Not relevant	
5	Post implantation loss	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect		In 1 /6 studies, weak effects were observed on post-implantation loss, however in the presence of strong maternotoxicity. Overall, no EAS-related adversity was observed with regards to the numbers of embryonic or foetal deaths and viable foetuses, litter viability as well as the number of live births.
6	Post implantation loss	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		
7	Post implantation loss	Rabbit	21d (GD 7-28)	Inhalation	15ppm	Change	n.s.s. ↓foetal viability, ↑early resorption, ↑post implantation in the presence of strong maternotoxicity: sign. ↓body weight gain (-154% d7-29) and ↓fc (-19% d7-29).	

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
8	Post implantation loss	Rabbit	21d (GD 7-28)	Inhalation	>12ppm	No effect	Range-finding study	
13	Post implantation loss	Rat	15d (GD 6-20)	Inhalation	>20ppm	No effect	Range-finding study	
14	Post implantation loss	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
5	Presence of anomalies*	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect		In 1 /6 studies, single anomalies are observed, however in the presence of strong maternotoxicity. Overall, no EAS-related adversity was observed with regards to the observed single anomalies.
6	Presence of anomalies*	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		
7	Presence of anomalies*	Rabbit	21d (GD 7-28)	Inhalation	15ppm	No effect	n.s.s. increase of single developmental findings (omphalocoele, vertebral centra anomaly, small spleen, 7th sternebra, irregular ossification of 6th sternebra, in the presence of strong maternotoxicity. Such effects are not considered EAS-related.	
8	Presence of anomalies*	Rabbit	21d (GD 7-28)	Inhalation	>12ppm	No effect	Range-finding study	
13	Presence of anomalies*	Rat	15d (GD 6-20)	Inhalation	>20ppm	No effect	Range-finding study	
14	Presence of anomalies*	Rat	16d (GD 6-20)	Inhalation	12ppm	**Increase**	n.s.s.↑ major blood vessel variation (single incidence) and reduced ossification of the 13th rib at top dose.	
10a	Reproduction	Rat	♂: 28 ♀: 58-65	Inhalation	>20ppm	No effect	Mating, fertility, conception, copulation indices not affected.	No EAS-related adversity was observed with regards to reproductive parameters.
11	Reproduction	Rat	16-19wk	Oral	>50ppm	No effect	Mating, fertility and fecundity index were not affected.	
5	Sex ratio	Rat	9d (GD 6-15)	Oral	>30mg/kg bw/day	No effect		No EAS-related adversity was observed with regards to sex ratio.
6	Sex ratio	Rabbit	12d (GD 6-18)	Oral	>10mg/kg bw/day	No effect		
7	Sex ratio	Rabbit	21d (GD 7-28)	Inhalation	>15ppm	Change	Very slight n.s.s. ↑♂/♀ ratio, in the presence of strong maternotoxicity: sign. ↓body weight gain (-154% d7-29) and ↓fc (-19% d7-29).	
10b	Sex ratio	Rat	30d	Inhalation	>20ppm	No effect		
11	Sex ratio	Rat	litter F1 & F2	Oral	>50ppm	No effect		

ID	Effect target	Species	Duration of exposure	Route of adminis-tration	Lowest Effect dose	Effect direction	Observed effect (+ and -)	Assessment of each line of evidence
12	Sex ratio	Rat	litter F1 & F2	Inhalation	>50ppm	No effect		
14	Sex ratio	Rat	16d (GD 6-20)	Inhalation	>12ppm	No effect		
5	Placental weight	Rat	9d (GD 6-15)	Oral	30mg/kg bw/day	Decrease	sign. ↓mean placental weight at top-dose, which is treatment-related. A ↓in placental weight is mainly induced by hypoplasia of the labyrinth zone, resulting from apoptosis and/or necrosis of trophoblasts, and usually leads to intrauterine growth retardation (IUGR). Whether this finding in isolation is EAS-related remains unclear, as this may also be related to strong maternotoxicity, with ↓body weight gains (corrected for uterine weight) amounting to -12, -15, -31% at resp. 3, 10, 30 mg/kg bw/day. Possibly, placental effects (via maternal toxicity or not) may be linked to hypoxia/GSH depletion, the latter overtly affected by both metam and MITC. A similar effect has been observed in one rat developmental assay with metam (although the opposite finding (placental weight increase) was observed in the rabbit developmental assay.	In one rat developmental study, decreased placental weight was observed, in the presence of strong maternotoxicity. Such effect may be explained by a GSH-depletion effect of MITC (as is the case for metam), and is unlikely to be EAS-related.
6	Placental weight	Rabbit	12d (GD 6-18)	Oral	-	n.d.		
7	Placental weight	Rabbit	21d (GD 7-28)	Inhalation	-	n.d.		
10b	Placental weight	Rat	30d	Inhalation	-	n.d.		
11	Placental weight	Rat	litter F1 & F2	Oral	-	n.d.		
12	Placental weight	Rat	litter F1 & F2	Inhalation	-	n.d.		
14	Placental weight	Rat	16d (GD 6-20)	Inhalation	-	n.d.		
12	Genital abnormalities	Rat	litter F1 & F2	Inhalation	> 21ppm	No effect	Abnormalities of genital organs were not observed.	No EAS-mediated adversity with respect to induction of genital abnormalities was observed.

*: external, visceral, skeletal; n.d.: not determined.

Note: parameters STBNDO in table 2.2.2.1-4 are potentially related to both T- and EAS modalities.

Assessment of the integrated line of evidence of fertility/developmental «sensitive to but not diagnostic of EATS» (*in-vivo*) parameters: further discussion and conclusions

Fertility/developmental parameters including time to meeting, gestation length, litter size or viability, number of live births, sex ratio were not affected by treatment with MITC.

RMS considers that changes of other fertility/developmental parameters, like litter/pup weight, numbers of embryonic/foetal deaths and viable foetuses, number of implantations, post-implantation loss or presence of anomalies, are more likely related to the general poor state of the animals or of the dams, and considering the toxicity of MITC, the findings are not of evident EAS-adversity. Effects on hormone-sensitive organs or tissues, like pituitary and adrenals were observed but in an inconsistent way and/or of insufficient magnitude and frequency to be considered toxicologically relevant or if of any relevance are not suggestive of any relationship to EATS. A potential effect on the placental weight, although measured in only one assay may be relevant, but taking into account the possible MoA of MITC (GSH depletion), is unlikely to be considered EATS-related.

In conclusion, in RMS opinion, there is no indication for endocrine-related adversity based on «sensitive to, but not diagnostic of EATS» parameters.

2.2.2.3 Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

Metam

EAS-mediated adversity has not been observed in any of the repeated dose toxicity studies conducted with metam in four species.

Potential EAS-mediated activity has been investigated in silico and in vitro and no indication for EAS-related endocrine activity was observed.

Table 2.10-8: WoE for EAS-mediated adversity: Metam

- EAS-mediated carcinogenicity in organs related to endocrine activity (e.g. testis, mammary gland, ovaries, uterus) was not observed in chronic/carcinogenicity studies in mouse and rat (study IDs 11, 12) which were conducted at the MTD.
- No EAS-related effect on fetal development (rat, rabbit) and fertility (rat) was observed (study IDs 13, 16, 17, 18, 37).
- No relevant effect on EAS-related parameters ("EATS-mediated" and "sensitive to but not diagnostic of EATS) was observed in repeated dose toxicity studies in rat (study IDs 4, 7, 8, 9, 18), mouse (study ID 6), rabbit (study ID 10), and dog (study IDs 2, 3). Observed organ weight changes (e.g. testes, epididymides, uterus, ovaries) are attributed to general systemic toxicity observed at these dose levels (for details refer to LoE Table).
- Systemic toxicity was identified by toxic effects to organs the following organs: liver, kidney, urinary bladder.
→ EAS-related adversity of metam is not observed.

Table 2.10-9: WoE for EAS-mediated endocrine activity: Metam

E-modality: - *in silico* The available in silico data provides supporting evidence that metam has no (anti)estrogenic activity based on QSAR model predictions including consensus predictions (for details refer to QSAR report [redacted], 2019, Appendix 1). - *in vitro* ToxCast ER prediction model is negative based on ToxCast/EDSP data (study ID 19).
A-modality: - *in silico* The available in silico data provides supporting evidence that metam has no (anti)androgenic activity based on QSAR model predictions including consensus predictions (for details refer to QSAR report; [redacted], 2019, Appendix 1). - *in vitro* ToxCast AR prediction model is negative based on ToxCast/EDSP data (study ID 20).
S-modality: - *in silico* The available in silico data provides supporting evidence that metam has no steroidal activity. However, only one model is available for steroid receptors: glucocorticoid receptor (GR). The results indicate low probability of binding (for details refer to QSAR report; [redacted], 2019, Appendix 1). - *in vitro* In vitro assays addressing potential effects on aromatase are negative (CompTox database; study IDs 21, 22).
→ EAS-related endocrine activity of metam is not observed.

MITC

EAS-mediated adversity has not been observed in any of the repeated dose toxicity studies conducted with MITC in four species.

Potential EAS-mediated activity has been investigated in silico and in vitro and no indication for EAS related endocrine activity was observed.

Table 2.10-10: WoE for EAS-mediated adversity: MITC

- EAS-mediated carcinogenicity in organs related to endocrine activity (e.g. testis, mammary gland, ovaries, uterus) was not observed in chronic/carcinogenicity studies in mouse and rat (study IDs 15-18). The MTD was exceeded in the inhalation carcinogenicity studies at the high dose both, for rat and mouse.
- No EAS-related effect on foetal development (rat, rabbit) and fertility (rat) was observed (study IDs 5-8, 10-14).
- No relevant effect on EAS-related parameters (EATS-mediated and "sensitive to but not diagnostic of EATS) was observed in repeated dose toxicity studies in rat (study IDs 2, 3, 10, 11, 12, 13, 14, 16, 19), mouse (study IDs 4, 15, 20), rabbit (study IDs 6-8), and dog (study ID 1). Observed organ weight changes (e.g. testes, epididymides, uterus, ovaries) are attributed to general systemic toxicity observed at these dose levels (for details refer to LoE Table).
- Mainly local effects on nose, trachea and lung were observed after inhalation exposure. Organ weight changes were observed (mainly liver, kidney, thymus, spleen) but no histopathological correlate. General toxicity showed consistent changes of body weight (gain), food consumption, clinical chemistry/haematology.
→ EAS-mediated adversity of MITC is not observed.

Table 2.10-11: WoE for EAS-mediated endocrine activity: MITC

E-modality: - *in silico* The available in silico data provides supporting evidence that MITC has no (anti)estrogenic activity based on QSAR model predictions including consensus predictions (for details refer to QSAR report; [redacted] 2019, Vol.3 B.6.8.3). - *in vitro* ToxCast ER prediction model is negative based on ToxCast/EDSP data (study ID 21).
A-modality: - *in silico* The available in silico data provides supporting evidence that MITC has no (anti)androgenic activity based on QSAR model predictions including consensus predictions (for details refer to QSAR report; [redacted], 2019, Vol.3 B.6.8.3). - *in vitro* ToxCast AR prediction model is negative based on ToxCast/EDSP data (study ID 22)
S-modality: - *in silico* The available in silico data provides supporting evidence that MITC has no steroidal activity. However, only one model is available for steroid receptors: glucocorticoid receptor (GR). The results indicate low probability of binding (for details refer to QSAR report; [redacted], 2019, Vol.3 B.6.8.3). - *in vitro* In vitro assays addressing potential effects on aromatase are negative (CompTox database; study IDs 23-25).
→ EAS-related endocrine activity of MITC is not observed.

2.2.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

Table 2.10-12: Selection of the relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EAS-mediated**" adversity	X (MITC)
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EAS-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "**EATS-mediated**" parameters. Depending on the outcome move to corresponding scenario	X (metam)
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.2.4 MoA analysis for EAS-modalities

Not applicable.

2.1.4.1 Postulate MoA

Not applicable.

2.1.4.2. Further information to be generated to postulate MoA

Not applicable.

2.1.4.3. Empirical support of the postulated MoA

Not applicable.

2.1.4.4. Conclusion on MoA analysis

Not applicable.

2.2.5 Conclusion of the assessment of EAS-modalities

According to the ECHA/EFSA ED Guidance, EAS-mediated adversity is sufficiently investigated if "all the 'EAS-mediated' parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation) or a two-generation reproductive toxicity study (OECD TG 416; test protocol according to latest version of January 2001)" are addressed.

Metam
Potential EAS-mediated adversity of metam was investigated in several repeated dose toxicity studies, including a two generation study, within which no EAS-mediated adverse effects were observed. However, as toxicity studies were performed according to OECD TG versions valid at study performance and as former OECD Test Guidelines did not address all EAS-relevant parameters as required in the ECHA/EFSA ED Guidance (i.e. vaginal opening, oestrus cycle, sperm parameters, balanopreputial separation, anogenital distance), EAS-mediated adversity is not sufficiently investigated.

As depicted in Figure 1 of the ECHA/EFSA ED Guidance, if EAS-mediated adversity was not observed but at the same time not sufficiently investigated, endocrine activity needs to be assessed. Potential endocrine activity with regards to E modality is considered sufficiently investigated as the available ToxCast ER Bioactivity Model is inactive.

The ToxCast AR Bioactivity Model is inactive for metam, however, this data is not sufficient according to ECHA/EFSA ED Guidance to investigate potential endocrine activity with regards to **A-mediated activity**.
Therefore, the following tiered approach according to chapter 3.4.3 Figure 3 of ECHA/EFSA ED Guidance is proposed: in a first instance OECD TG 458 (*In vitro* androgen transactivation assay- OECD TG 458) is conducted. If this test is negative, a Hershberger Assay (OECD TG 441) is proposed.

With regards to potential **S-mediated activity** ToxCast/EDSP data is available for aromatase which showed no activity of metam. For sufficient investigation of potential S-related endocrine activity according to chapter 3.4.3 Figure 3 of ECHA/EFSA ED Guidance, the following level 2 assays are proposed to be conducted with metam: H295R steroidogenesis assay (OECD TG 456) and the aromatase assay (human recombinant) (OPPTS 890.1200).

In conclusion, no EAS-mediated adversity and activity was observed for metam, providing no indication for endocrine disrupting properties of the active substance. According to ECHA/EFSA ED Guidance EAS-mediated adversity was not sufficiently investigated and thus, endocrine activity needs to be addressed. Potential E-related endocrine activity was not observed and is considered sufficiently investigated.
The A- and S-related endocrine activity was not observed but is not sufficiently investigated based on the requirements of the ECHA/EFSA ED Guidance.

Following discussion with the RMS Belgium during a meeting on endocrine disruption, the notifier decided to conduct the following studies for human health:

- *In vitro* androgen transactivation assay (OECD TG 458)
- *In vivo* Hershberger assay (OECD TG 441with extension to T-parameters, only if OECD TG 458 is negative)
- *In vitro* H295R steroidogenesis assay (OECD TG 456)
- *In vitro* aromatase assay (human recombinant) (OPPTS 890.1200)

The results of these studies, if acknowledged during the Peer Review and if requested by EFSA, will be evaluated during the Stop-of-the-Clock (SoC).

MITC
Potential EAS-mediated adversity of MITC was investigated in several repeated dose toxicity studies, including two two-generation reproductive toxicity studies, within which no EAS-mediated adverse effects were observed. One of the two-generation reproductive toxicity studies was performed according to OECD TG version prior to 2001 and thus did not address all EAS-relevant parameters as required in the ECHA/EFSA ED Guidance (e.g. vaginal opening, oestrus cycle, sperm parameters, balanopreputial separation). However, the second two-generation reproductive toxicity was performed according to OECD TG 416 (2001) investigating the relevant parameters as referenced in the ECHA/EFSA ED Guidance. Therefore, potential EAS-mediated adversity of MITC is considered sufficiently investigated.

Potential endocrine activity with regards to E modality is considered sufficiently investigated according to ECHA/EFSA Guidance as the available ToxCast ER Bioactivity Model is inactive. With regards to potential S-mediated activity ToxCast/EDSP data is available for aromatase which showed no activity of MITC. No in vitro mechanistic data are available for steroidogenesis and therefore, potential endocrine activity related to S-modality is considered not sufficiently investigated. However, since no EAS-mediated adversity was observed, which is sufficiently investigated, scenario 1a (Table 5, ECHA/EFSA ED Guidance) applies and the ED criteria are not met. Therefore, no further studies to investigate A- and S-related activity need to be performed.

In conclusion, no EAS-mediated adversity (sufficiently investigated) and activity were observed for MITC, providing no indication for endocrine disrupting properties of the substance. Moreover, as depicted in Figure 1 of the ECHA/EFSA ED Guidance, if EAS-mediated adversity is not observed when sufficiently investigated, the ED criteria are not met.

2.3 Overall conclusion on the ED assessment for humans

According to the ED criteria laid down in Regulation (EU) 2018/605 for identification of a substance as an endocrine disruptor, the following criteria need to be fulfilled: identification of an adverse effect and endocrine activity as well as a biological link between those two.

Metam
T-mediated adversity of metam has been sufficiently investigated within repeated dose toxicity studies in 4 species. Thyroid weight was decreased in 1 study only with no correlating histopathological changes and at a dose where general systemic toxicity was apparent.
According to the ED criteria, endocrine mediated adversity as well as activity and the biological link between those two must be given to identify a substance as an endocrine disruptor. Since metam does not induce T-mediated adversity, which is sufficiently investigated based on a weight of the evidence approach, the ED criteria are not met with regards to T-modality.

EAS-mediated adversity was not observed for metam but is not considered sufficiently investigated according to requirements of the ECHA/EFSA ED Guidance since specific requested parameters were not included in former OECD TG which were applicable at study performance.
E-related endocrine activity is considered sufficiently investigated based on in silico and in vitro mechanistic data (including the ToxCast ER Bioactivity Model) which showed no endocrine activity of the active substance. Available in silico and in vitro data (including the ToxCast ER Bioactivity Model) on A-related endocrine activity is negative. However, according to ECHA/EFSA ED Guidance, A-related potential endocrine activity is not sufficiently investigated. Information on S-related endocrine activity is available from CompTox database for aromatase inhibition which is negative. However, according to ECHA/EFSA ED Guidance, this is not considered sufficiently investigated.

Following discussion with the RMS Belgium during a meeting on the endocrine disruptive potential of metam, the notifier decided to conduct the following studies for human health:
- ***In vitro*** **androgen transactivation assay (OECD TG 458)**
- ***In vivo Hershberger assay (OECD TG 441with extension to T-parameters, only if OECD TG 458 is negative)***
- ***In vitro*** **H295R steroidogenesis assay (OECD TG 456)**
- ***In vitro*** **aromatase assay (human recombinant) (OPPTS 890.1200)**

In conclusion, metam does not meet the ED criteria for EATS modalities, as neither adversity nor endocrine activity were observed. It is acknowledged that A- and S-related activity is not sufficiently investigated according to the ECHA/EFSA ED Guidance which will be addressed in the new studies.

MITC
T-mediated adversity of MITC has been sufficiently investigated according to ECHA/EFSA Guidance within repeated dose toxicity studies in four species. Thyroid weight changes were observed in repeated dose toxicity studies in mouse and rat. However, thyroid weight changes were not consistent and no corresponding histopathological changes were observed. Therefore, no relevant T-mediated adversity was observed for MITC.
Since MITC does not induce T-mediated adversity, which is sufficiently investigated based on a weight of the evidence approach, the ED criteria are not met with regards to T modality.

Potential EAS-mediated adversity of MITC was investigated in several repeated dose toxicity studies, including two two-generation reproductive toxicity studies. One two-generation reproductive toxicity study was performed according to OECD TG 416 (2001) investigating the relevant parameters as referenced in the ECHA/EFSA ED Guidance. Therefore, potential EAS-mediated adversity of MITC is considered sufficiently investigated.
Since no EAS-mediated adversity was observed after MITC treatment, the ED criteria with regards to EAS modalities are not met.

In conclusion, MITC does not meet the ED criteria for EATS modalities and is thus not considered an endocrine disruptor.

3. ED assessment for non-target organisms

The assessment follows the strategy as laid down in the new ECHA/EFSA ED Guidance. The available basic studies with non-target organisms on metam and its degradation product methyl isothiocyanate (MITC) are meeting the data requirements for ecotoxicology.

Since the effects measured in these studies cannot be assigned to a specific modality (E, A, T or S) and were all ranked as "sensitive to, but not diagnostic of EATS" or "systemic toxicity", the subsequent ED assessment including the table on the lines of evidence for EAS- and T-modalities is not presented separately, but jointly in section 3.1.

3.1. ED assessment for T-modality

The information summarized in Appendix E and the resulting data matrix is considered as reliable and relevant. The studies are conducted according to GLP and recent guidelines. None of the relevant published studies have been considered reliable and they were therefore not included into Table E and the Lines of Evidence. The rationale for relevance and reliability is included in the literature evaluation documentation (please refer to the related Literature Review Report).

All effect data are assigned as "sensitive but not diagnostic of EATS" or "systemic toxicity".

Studies on the chronic toxicity to fish

A 28-day study (according to OECD 204) was performed with *Oncorhynchus mykiss* exposed to MITC (study ID 36). The threshold level for lethal effects was > 0.01 and < 0.02 mg/L, and for signs of intoxication like convulsions as well as for the development of body weight and length it was > 0.005 and < 0.01 mg/L (nominal concentrations). As a conclusion, effects on growth and sublethal effects occurred at concentrations slightly below at which overt toxicity (effects on survival) was observed, hence these effects are not indicative of endocrine mediated mechanisms.

Another 33-day chronic fish study was performed with *Pimephales promelas* according to OECD 210 (study ID 37). The mortality based EC_{10} value was 0.00929 mg/L (mean measured). Effects on length and weight were observed at test concentrations where mortality already occurred (EC_{10} wet weight: 0.0142 mg/L) indicating that these effects can be seen a result of secondary consequences of general systemic toxicity. Decreased hatching success was noted at concentrations above effects on survival, thus these can also be considered as secondary effects of systemic toxicity and not relevant for the identification of potential endocrine disrupting properties.

In general, invertebrates are not considered to be in the scope of the new ECHA/EFSA ED Guidance, which relates only to fish, amphibians and birds. However, for the sake of completeness, available invertebrate data are reported here as well.

Studies on the chronic toxicity to aquatic invertebrates:

The chronic toxicity of MITC to *Daphnia magna* was investigated according to OECD 202, Part II (study ID 38). There were statistically significant decreases in survival and reproduction at 25 µg/L and higher concentrations. The NOEC was 12.5 µg/L. In conclusion, the observed effects can be considered as evidently not endocrine-mediated, but more be indicative of general toxicity as effects on reproduction occurred at concentrations where systemic toxicity and mortality were observed.

As conclusion from the available studies, no indications for an endocrine disrupting potential can be derived from the observed effects.

3.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

In fish and daphnids, effects were only seen at MITC concentration ranges where systemic effects occur (mortality), and thus can be classified as secondary effects (Table 2.10-13).
The ED Guidance specifies that "adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor". According to this, the top dose selected for ecotoxicological testing shall be set at the maximum tolerated concentration (MTC). As cited in the ED Guidance, "the MTC is defined as the highest test concentration of the chemical which results in less than 10% mortality", and further: "where potentially endocrine-related adverse effects are only observed at excessive toxic dose/concentration (i.e. only observed above the MTD or MTC) they should not be considered indicative of endocrine disruption". Therefore, any adverse effects observed are considered to be a consequence of excessive toxicity and cannot be attributed to endocrine related modalities.

Table 2.10-13: Lines of evidence for adverse effects and endocrine activity related to EATS-modality for MITC

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
36a	Systemic toxicity	Mortality	*Oncorhynchus mykiss*	28	Days	Uptake from water	0.02	mg/L	Increase	Decrease in length and weight, hatching success, change in behavior and convulsions/ spasms in a concentration range where systemic effects (increase of mortality) already occur	In fish: no effects or effects only in a concentration range where systemic effects occur (mortality) - secondary effects	Adversity: Only secondary effects as a consequence of systemic toxicity
36b	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Oncorhynchus mykiss*	28	Days	Uptake from water	0.01	mg/L	Decrease			
36c	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Oncorhynchus mykiss*	28	Days	Uptake from water	0.01	mg/L	Decrease			
36d	[Not in list]	Convulsions, spasms, lying on the bottom, discoloration	*Oncorhynchus mykiss*	28	Days	Uptake from water	0.01	mg/L	Increase			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
36e	[Not in list]	Apathy, swimming near the bottom	*Oncorhynchus mykiss*	28	Days	Uptake from water	0.02	mg/L	Increase			
37a	Systemic toxicity	Mortality	*Pimephales promelas*	33	Days	Uptake from water	0.0163	mg/L	Increase			
37b	Sensitive to, but not diagnostic of, EATS	Hatching success	*Pimephales promelas*	33	Days	Uptake from water	0.0334	mg/L	Decrease			
37c	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Pimephales promelas*	33	Days	Uptake from water	0.0163	mg/L	Decrease			
37d	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Pimephales promelas*	33	Days	Uptake from water	0.0163	mg/L	Decrease			
38a	Sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	*Daphnia magna*	21	Days	Uptake from water	0.025	mg ai/L	Decrease	Decrease in reproduction only at highest concentration where systemic effects occur (decrease in survival)	In Daphnia: effects at concentrations where mortality already occur	
38b	Systemic toxicity	Mortality	Daphnia magna	21	Days	Uptake from water	0.025	mg ai/L	Increase			

3.1.1.1. Assessment of the integrated lines of evidence and weight of evidence

Regarding the assessment of potential EATS-mediated adversity of metam and MITC, only secondary effects as a consequence of systemic toxicity are observed. The effects are ranked as "sensitive to, but not diagnostic of EATS modalities" and "systemic toxicity".

As depicted in Figure 1 of the ECHA/EFSA ED Guidance, if EATS-mediated adversity was not observed but at the same time not sufficiently investigated, endocrine activity needs to be assessed. T-mediated activity and potential EAS-mediated activity have been investigated *in silico* and *in vitro*. Neither an indication for thyroid receptor (TR) activation nor for EAS-related endocrine activity is provided from in *silico* or *in vitro data* (see section 2, human health).

Thus, in conclusion from the available data, no indication for (potentially) EATS-mediated adverse effects nor for endocrine activity can be derived.

3.1.2 Initial analysis of the evidence and identification of the relevant scenario

The studies on metam and MITC submitted by the notifier were conducted in accordance with the data requirements laid down in Regulation (EC) No 1107/2009 and were performed according to the relevant US EPA and/or OECD testing guidelines in their contemporary versions.

With regard to effects on <u>non-target organisms</u>, studies with fish and an invertebrate species are available. Toxicity data from these studies as well as *in silico* screening and data from ToxCast indicate no concern for adversity and endocrine activity. However, it has to be stated that formally when strictly following the ED Guidance, the EATS-mediated parameters have not been sufficiently investigated. To have the EATS-mediated parameters "sufficiently investigated" according to the ED Guidance, a MEOGRT (OECD 240, Medaka Extended One Generation Reproduction Test) and a LAGDA (OECD 241, Larval Amphibian Growth and Development Assay**)** would be required which are highly time-, cost- and animal-intensive studies. Further, to have endocrine activity "sufficiently investigated", an FSTRA (OECD 229, Fish Short Term Reproduction Assay) and an AMA (OECD 231, Amphibian Metamorphosis Assay) would be required which are also studies not conducted in general for the dossier preparation.

Regarding the assessment of potential endocrine activity for non-target organisms, no studies analysing parameters labelled as "*in vivo* mechanistic" are available. As stated in the ECHA/EFSA ED Guidance, the available fish early life stage test (study ID 37, OECD TG 210) is not designed to give information on endocrine effects, but should be considered as it provides information on both general toxicity (information which is necessary for a reliable interpretation of potential endocrine disrupting properties) and on parameters that might be sensitive to EATS mediated parameters (such as hatchability and development). This study alone is, however, not considered sufficient to conclude on EATS mediated activity or adversity.

Hence, the questions as stated in the flowchart of the ED Guidance "Have the EATS-mediated parameters been sufficiently investigated?" and "Has EATS-mediated activity been sufficiently investigated?" would in this case need to be answered with "no" (compare Table 2.10-14). Based on the available data, **scenario 2a iii** from Table 5 of the ECHA/EFSA ED Guidance would apply here (Table 2.10-14). Thus further studies might be considered necessary since only three studies with non-target organisms are available.

Still, based on the available data, metam and MITC did not exert adverse effects on the endocrine system in relevant animal models, and the available data also do not show relevant endocrine activity. So in general, there is no reasonable alert nor evidence for potential effects of metam and MITC on oestrogen, androgen, steroidogenesis or thyroid pathways.

<u>Weight of evidence evaluation:</u>

Apart from an assessment following the strategy outlined in the new ECHA/EFSA ED Guidance, a weight of evidence evaluation of all available data is considered appropriate as the guidance also clearly states that the evaluation should be based on all available relevant scientific data (in vivo studies or adequately validated alternative test systems predictive of adverse effects in humans or animals; as well as in vivo, in vitro or, if applicable, in silico studies informing about endocrine modes of action).

Thus basically, the ECHA/EFSA ED Guidance does in principle not aim at the generation of new data, but instead it emphasises that the assessment should be based on the available database, with regard to the respective underlying regulatory program.

In general, for the bulk of active substances which have been evaluated or which are currently under evaluation, there will be an identical "lack of data", *i.e.* a limited dataset available which could be considered as "not sufficient

for a conclusion regarding ED", as the related data requirements were not available at the time of the initial submission.

From a more general point of view, the consequence of this situation would be to request a vast number of highly animal-intensive vertebrate fish and amphibian studies for any substance and/or product authorisation process. This represents a scenario which is clearly contrasting with ethical and animal welfare claims supported by the European Member States.

Thus, further vertebrate studies on non-target organisms justified by a supposed lack of sufficient data should not be requested to conclude on potential endocrine disrupting properties. For sure, in cases where there is any observed alert or hint for endocrine disrupting properties, this concern should be further assessed or investigated.

As pointed out, the conducted literature search for metam and MITC as outlined above, has shown no relevant and reliable hits nor concern with regard to any probable alert regarding endocrine disrupting properties in non-target organisms.

Furthermore, there is data investigating endocrine activity from a comprehensive battery of testing on Level 2 of the OECD Conceptual Framework. This data is available from CompTox data base, covering potential EATS modalities, and shows no alerts regarding endocrine activity (no or only low activity predictions, see section 2, human health). This is confirmed by a specific ED QSAR profiling, *i.e. in silico* screening ([redacted] 2019).

In addition, there were no indications for adverse effects of metam and MITC in the overall assessment. Following the new ECHA/EFSA ED Guidance, even if the assumption would be "EATS-mediated parameters on adversity not sufficiently investigated", and given that there are "no indications for endocrine activity", this would be sufficient to conclude on "ED criteria not met".

In conclusion, the overall assessment of the available data (*in silico*, *in vitro* and *in vivo* toxicological and ecotoxicological studies regarding adversity and endocrine activity) should be sufficient to give a complete picture in a weight of evidence approach to exclude a concern regarding the adversity of metam and MITC.

Table 2.10-14: Selection of the relevant scenario for NTO

Adversity based on EATS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EATS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EATS-mediated endocrine activity** observed	*(x) (metam, MITC) (opinion notifier)*
No (not sufficiently investigated)	**No (not sufficiently investigated)**	**2a (iii)**	**Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario**	**x (metam, MITC) (opinion RMS)**
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

When strictly following the ECHA/EFSA ED Guidance for the case that remaining uncertainties would require animal testing, despite of the weight of evidence argumentation presented above, the following testing strategy is proposed:

In any case, testing on endocrine activity should be the first step, i.e. FSTRA (OECD 229, Fish Short Term Reproduction Assay) in combination with AMA (OECD 231, Amphibian Metamorphosis Assay). It has to be kept

in mind that these studies are time-, cost- and especially animal-intensive, given that there is not alert at all from the available data. This should be seen from a perspective of animal welfare as well.

An alternative approach for testing endocrine activity would be to start with a screening battery testing on endocrine activity, i.e. XETA (Xenopus embryonic thyroid signalling assay, OECD 248), EASZY (Detection of Substances Acting through Estrogen Receptors using Transgenic cyp19a1b GFP Zebrafish Embryos, draft OECD TG) and RADAR (Rapid androgen disruption adverse outcome reporter assay, draft OECD TG) assays to gain faster information with less animal consumption. In case no activity is derived from these assays, no further testing is deemed necessary. If endocrine activity is observed, it has to be discussed which further studies might be required to draw a firm conclusion on potential endocrine activity, or EATS-mediated parameters.

3.1.3 MoA analysis for T-modality

Not applicable

3.1.4 Conclusion on the ED assessment for T-modality

Adverse effects observed in the ecotoxicological studies available for metam and MITC were associated with general toxicity, and according to the ED criteria, "adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor with respect to non-target organisms". Available data from CompTox data base investigating endocrine activity from a comprehensive battery of testing on Level 2 of the OECD Conceptual Framework shows no alerts regarding endocrine activity (no or only low activity predictions, see section 2, human health). However, A- and S-related endocrine activity was not observed but is not sufficiently investigated based on the requirements of the ECHA/EFSA ED Guidance (see section 2 human health).

Furthermore, only three ecotoxicological studies (data requirements) are available. Therefore further vertebrate studies on non-target organisms, a FSTRA (OECD 229, Fish Short Term Reproduction Assay) and an AMA (OECD 231, Amphibian Metamorphosis Assay), justified by a supposed lack of sufficient data might be considered as helpful to conclude on potential endocrine disrupting properties of metam. It is recommended to conduct these tests with metam only (not MITC) in order to relate the effects to a realistic exposure scenario with consideration of all possible metabolites.

Following discussion with the RMS Belgium during a meeting on the endocrine disruptive potential of metam, the proposed weight of evidence approach was not considered sufficient. The RMS Belgium advised against conducting embryonic testing as these tests are not fully accepted at European level.
For this reason, the notifier will initiate the following studies for non-target organisms:

- *In vivo* **fish short term reproduction assay (OECD 229)**
- *In vivo* **amphibian metamorphosis assay (OECD 231)**

The studies will be conducted with metam only (not MITC) in order to relate the effects to a realistic exposure scenario with consideration of all possible metabolites.

Update 08.02.2022: The above studies for non-target organisms have been initiated in the meantime by the notifier and are expected to be finalised in Q1 2021 as announced.

3.2 ED assessment for EAS-modality

Since the effects measured in these studies cannot be assigned to a specific modality (E, A, T or S) and were all ranked as "sensitive to, but not diagnostic of EATS" or "systemic toxicity", the subsequent ED assessment including the table on the lines of evidence for EAS- and T-modalities is not presented separately, but jointly in section 3.1.

Assessment and conclusion by the RMS:

Evaluation for Human health:

Conclusion of the assessment of T-modality

Metam

T-mediated adversity of metam has been sufficiently investigated within 9 repeated dose toxicity studies (4 and 9 studies addressing thyroid weight and histopathology, respectively) with rat, mouse, rabbit, and dog. The only effect observed was a decrease in thyroid weights in one subacute rat inhalation study (21- day exposure) with no corresponding histopathological changes. Therefore, no relevant T-mediated adverse effects were observed for metam.

With regards to T-mediated endocrine activity *in silico* and *in vitro* mechanistic data are available based on which there is no evidence that metam might interact with the thyroid receptor. Information on a potential inhibition of TPO is equivocal. However, ethylene thiourea (ETU), the relevant metabolite of ethylene bis[dithiocarbamates] (EBDCs) responsible for TPO inhibition, cannot be formed by metam based on its chemical structure. RMS considers that indeed the ETU-type of thiocarbamates are poorly comparable with the dithioarbamates, thus this information is not relevant.

According to the ED criteria laid down in Regulation (EU) 2018/605, endocrine mediated adversity as well as activity and the biological link between those two must be given to identify a substance as an endocrine disruptor. Since metam does not induce T-mediated adversity, which is sufficiently investigated based on a weight of the evidence approach, the ED criteria are not fulfilled.

However, notifier indicated that, if a Hershberger Assay (OECD TG 441, study announced) is considered necessary for investigation of A-related activity, thyroid hormone measurements will be included in the study design to substantiate the weight of evidence approach on T-related activity.

MITC

T-mediated adversity of MITC has been sufficiently investigated within 6 repeated dose toxicity studies with rat, mouse, rabbit, and dog. The only effect observed were changes in thyroid weight in repeated dose toxicity studies in mouse and rat. However, thyroid weight changes were not consistent and no corresponding histopathological changes were observed. Therefore, no relevant T-mediated adverse effects were observed for MITC.

With regards to T-mediated endocrine activity *in silico* and *in vitro* mechanistic data are available based on which there is no evidence that MITC might interact with the thyroid receptor. *In silico* information on potential inhibition of TPO is inconclusive. Notifier highlighted that here are only a few models available for thyroid receptor. While a molecular docking method indicates low probability of binding, two models available in the Danish QSAR database yield positive results for Metam and inconclusive results for the metabolite MITC. Both compounds are present in the training sets of the models, and the experimental value is positive in case of Metam (as well as for two similar substances, Metam Potassium and Methylcarbamic Acid), and it is negative in case of MITC. While without further evidence, the overall outcome of the *in silico* screening on thyroid activity is thus considered to be inconclusive, it is of note that no meaningful thyroid findings were observed for neither metam and MITC. In isolation, such *in-silico* data are regarded to provide complementary information, but are not further considered to impact on the hazard assessment for ED effects, in the absence of any meaningful effect of the a.s. on the thyroids *in-vivo*.

According to the ED criteria laid down in Regulation (EU) 2018/605 for identification of a substance as an endocrine disruptor, the following criteria need to be fulfilled: identification of an adverse effect and endocrine activity as well as the biological link between those two.

MITC does not induce thyroid-related effects, which is sufficiently investigated based on a weight of the evidence approach, therefore, the ED criteria are not fulfilled (Scenario 1a).

In conclusion, the ED criteria for MITC are not met with regards to T-modality.

Conclusion of the assessment of EAS-modalities

Metam

Parameters for EAS-mediated **adversity** were investigated in repeated dose toxicity studies including a 2-Generation study according to OECD Guideline versions applicable at study performance (prior to 2001). As former OECD Test Guidelines did not address all ED relevant parameters as required in the ECHA/EFSA ED Guidance, the following parameters were not investigated:

- age at vaginal opening
- oestrus cyclicity
- sperm parameters
- age at balanopreputial separation
- anogenital distance

Therefore, **RMS** is of the opinion that EAS-mediated adversity is ***not sufficiently investigated*** for metam according to ECHA/EFSA ED Guidance.

EAS-mediated **activity**:
E: Output data from the ToxCast ER Bioactivity Model is available and thus, E-mediated activity is considered sufficiently investigated according to the ED Guidance.
A: Output data from the ToxCast AR Bioactivity Model is available which is negative. However, according to the ED Guidance, the **AR Bioactivity model is not sufficient for A-related activity investigation**.
Therefore, a tiered testing approach according to the ED Guidance is proposed including

- an Androgen Transactivation Assay (OECD TG 458) and a
- Hershberger Assay (OECD 441) + T-parameters if OECD TG 458 is negative.

S: ToxCast/EDSP data on aromatase are available. For sufficiently investigating potential steroidogenic activity, the following tests according to chapter 3.4.1 of the ECHA/EFSA ED Guidance are proposed:

- H295R steroidogenesis assay (OECD TG 456) and
- the aromatase assay (human recombinant, OPPTS 890.1200).

It is noted that, in the foetal development (rat, rabbit) studies with metam, various effects were identified which lead to the classification of metam as a developmental toxicant. However, the findings are related to structural adverse effects at the top-dose, for which association with severe maternotoxic toxicity is likely. The observed growth/ossification retardation can also not unequivocally be related to EAS effects.

MITC
EAS-mediated **adversity**:
Parameters for EAS-mediated adversity were investigated in repeated dose toxicity studies including two two-generation reproductive toxicity studies according to OECD Guideline versions applicable at study performance. One 2-Generation study was performed according to OECD TG 416 (2001), investigating all relevant EATS-related parameters as referenced in the ECHA/EFSA Guidance (Table 14).
Therefore, **EAS-mediated adversity of MITC is sufficiently investigated** according to ECHA/EFSA ED Guidance.

EAS-mediated **activity**:
E: Output data from the ToxCast ER Bioactivity Model is available, and thus**, E-mediated activity is considered sufficiently investigated.**
A: Output data from the ToxCast AR Bioactivity Model is available which is negative. According to the ECHA/EFSA ED Guidance, A-related endocrine activity is not sufficiently investigated. However, since adversity was not observed, although "sufficiently investigated" according to chapter 3.4.1 of the ECHA/EFSA ED Guidance, **no further tests addressing A-related endocrine activity are considered necessary**.
S: ToxCast/EDSP data on aromatase are available which are negative. According to the ECHA/EFSA ED Guidance the tests as referred to in chapter 3.4.3 Table 4 (H295R steroidogenesis assay (OECD TG 456) and aromatase assay (human recombinant, OPPTS 890.1200) need to be available for sufficient S-related activity investigation.
However, since EAS-mediated adversity was not observed and considered "sufficiently investigated" according to chapter 3.4.1 of the ECHA/EFSA ED Guidance, **no further testing for S-related endocrine activity of MITC is required** by the notifier.

It is of note that in the inhalation 2-generation study with MITC, no apical reprotoxicity parameters were severely affected. However, reprotoxicity NOAEL was prudently based upon increased oestrous cycle and gestation length (F_0,F_1), and delayed vaginal patency (F_1). The effects were observed at doses which were already toxic for the parental generation, thus overall, it seems unlikely that MITC should, just like metam itself be regarded an overt reprotoxicant.

In conclusion the notifier decided to conduct the following studies for human health on the a.s. **metam**:

- *In vitro* androgen transactivation assay (OECD TG 458)
- *In vivo* Hershberger assay (OECD TG 441, only if OECD TG 458 is negative)
- *In vitro* H295R steroidogenesis assay (OECD TG 456)

- *In vitro* aromatase assay (human recombinant) (OPPTS 890.1200).

These data are awaited and should be submitted, in order to conclude definitively on the ED properties of metam, and indirectly of its main metabolite MITC.

General conclusion of RMS:

For **MITC**, on the basis of existing data, **scenario 1a** is identified; MITC does not meet the ED criteria.

For **metam**, on the basis of existing data on the T-modality**, scenario 1a** is identified; MITC does not meet the ED criteria for thyroidal effects.

For **metam**, the adversity based on EAS-mediated parameters is insufficiently investigated and mechanistic OECD CF level 2/3 test should be performed.
Consequently, **scenario 2a (iii)** is identified for EAS-modalities.
The notifier agreed to conduct the following studies for human health on the a.s. **metam**:

- *In vitro* androgen transactivation assay (OECD TG 458)
- *In vivo* Hershberger assay extended with T-parameters (OECD TG 441, only if OECD TG 458 is negative)
- *In vitro* H295R steroidogenesis assay (OECD TG 456)
- *In vitro* aromatase assay (human recombinant) (OPPTS 890.1200).

These data are awaited and should be submitted, in order to conclude definitively on the ED properties (EAS-modalities) of **metam**.

RMS: to date (February 2022), the planned additional studies mentioned hereabove (**OECD TG 458**, **OECD TG 456**, **OPPTS 890.1200** and, potentially, **OECD TG 441**) are not available. As a consequence, A complete picture of the ED landscape may not be obtained yet, hence a definitive conclusion on ED may not be drawn.
See also section 3.1.4 : list of studies to be generated, still ongoing or available but not peer reviewed.

Evaluation for Non-Target Organisms:

The assessment provided by the notifier in this position paper is generally in line with the ECHA/EFSA Guideline. The RMS agrees with the lines of evidence as presented in Table 2.10-13, and their interpretation.

In relation to the literature search, it is claimed in the position paper that the 9 publications that were found in the literature review with potential relevance for ED properties were evaluated with regard to their reliability, and that none of these publications were reliable. This is however not correct. According to the literature review reports, summarised in Volume 3 (CA) Section B.9.11, these studies were not assessed for their reliability, but only for their relevance. They were excluded for further assessment based on the following relevance criterion:
"Relevant for the evaluation of potential ED properties (results on EATS (oestrogen, androgen, thyroid, steroidogenic) mediated adverse effects and/or endocrine activity)"
These publications were excluded because no EATS-related parameters were assessed (e.g. some studies were mechanistic studies, which investigated the relation between metam sodium exposure and notochord distortions and craniofacial abnormalities in zebrafish), or because MITC was tested in combination with another substance.

As correctly stated by in the position paper, the dataset available is very limited and only endpoints "sensitive to, but not diagnostic of EATS modalities" were investigated. Therefore, the available dataset is not sufficient to conclude on EATS-mediated activity or adversity. According to the ECHA/EFSA guidance document, this corresponds to scenario 2a (iii), which means that further data needs to be generated to enable a conclusion.

In the position paper, a further "weight of evidence assessment" is presented, in which it is argued that, despite the outcome of the assessment according to the ECHA/EFSA Guidance Document, it should not be necessary to generate further data, and that the is no concern for endocrine disruptive effects of metam and MITC. However, the RMS considers the weight of the available evidence too weak to agree with this conclusion.

In addition, the following is noted: In the position paper, it is stated that "*based on the available data, metam and MITC did not exert adverse effects on the endocrine system in relevant animal models*". It should however be noted that the available studies discussed in the assessment were only studies performed with MITC, and no relevant data for metam itself is available.

The RMS agrees that in principle, the ECHA/EFSA Guidance Document does not aim at the generation of new data. However, if data is lacking to enable a conclusion, the same guidance document also indicates that further data needs to be generated, in line with the testing strategy.

The notifier notes that for the bulk of the active substances currently under evaluation, the situation would be similar to that of metam and MITC (*i.e.* the dataset available is not sufficient to conclude), and that if for all these substances additional data would be requested, a high number of vertebrate studies would need to be generated, which is in contrast with animal welfare and the commitment to reduce animal testing. While this is acknowledged, and the RMS agrees that vertebrate testing should be minimised as much as possible. However, there is a legal obligation to investigate endocrine disrupting properties of an active substance. In that respect, the situation of other active substances is not really relevant.

The ED assessment was discussed in a dedicated meeting between the RMS and one of the notifiers (Taminco) on 17-09-2019. As indicated in the position paper above, it was already communicated at that meeting that the presented weight of evidence was not considered sufficient by the RMS. The notifier also mentions in the position paper that the RMS advised against conducting embryonic testing. This was because the test protocol of the RADAR and EASZY assay is still in the draft OECD guideline stage, and therefore these assays are not yet implemented in the testing strategy of the ECHA/EFSA Guidance Document. At the time of that meeting, the OECD test guideline for the XETA assay was already published. However, the discussion on how to implement this test in the testing strategy of the ECHA/EFSA Guidance document was still ongoing. Therefore, the outcome of an assessment based on these embryonic assays was highly uncertain.

The RMS agrees with the testing strategy for **metam** proposed by the notifier (i.e. perform:

- an *in vivo* fish short term reproduction assay (OECD 229) and an
- *in vivo* amphibian metamorphosis assay (OECD 231).

For **MITC, no specific studies are proposed by the notifier**. The intended studies will be conducted wih **metam** only, in order to relate the effects to a realistic exposure with consideration of all possible metabolites.

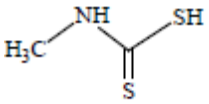
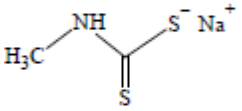
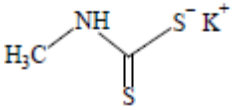
The updated version of this position paper mentions that these studies were initiated and were expected to be finalised in Q1 2021. However, to date, these studies have not been submitted to the RMS. A final conclusion on the potential endocrine disrupting effects of metam is therefore not possible.
See also section 3.1.4 : list of studies to be generated, still ongoing or available but not peer reviewed.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 69: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*[The Guidance for identification and naming of substances under REACH and CLP can be found at the following link: http://echa.europa.eu/guidance-documents/guidance-on-reach]* Methyldithiocarbamic acid Variant 1: sodium-N-methyldithiocarbamate Variant 2: potassium-N-methyldithiocarbamate
Other names (usual name, trade name, abbreviation)	Metam Variant 1: metam-sodium, metham-sodium, metham-Na Variant 2: metam-potassium, metham-potassium, metham-K
ISO common name (if available and appropriate)	Metam (incl. –sodium and – potassium)
EC number (if available and appropriate)	205-632-2 Variant 1: 205-293-0 Variant 2: 205-292-5
EC name (if available and appropriate)	-
CAS number (if available)	144-54-7 Variant 1: 137-42-8 Variant 2: 137-41-7
Other identity code (if available)	CIPAC: 20 Variant 1: 20.011 Variant 2: 20.019
Molecular formula	$C_2H_5NS_2$ Variant 1: $C_2H_4NNaS_2$ Variant 2: $C_2H_4KNS_2$
Structural formula	 Variant 1:  Variant 2: 
SMILES notation (if available)	CNC(=S)S CNC(=S)[S-].[Na+] CNC(=S)[S-].[K+]
Molecular weight or molecular weight range	107.2 g/mol Variant 1: 129.2 g/mol Variant 2: 145.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable: Metam has no stereoisomers

Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable: Metam is not an UVCB substance
Degree of purity (%) (if relevant for the entry in Annex VI)	**Annex I - according to COMMISSION IMPLEMENTING REGULATION (EU) No 359/2012 of 25 April 2012:** Technical concentrate (TK): **metam-sodium TK**: min. 400 g/kg – max. 442 g/kg **metam-potassium TK:** min. 520 g/kg – max. 560 g/kg Dry weight basis (calculated): **metam-sodium:** min. 965 g/kg **metam-potassium:** min. 990 g/kg **AIR 5 (Taminco BV):** Dry weight basis (calculated, modified equation to derive TC from TK values, refer to Vol. 4): **metam-sodium:** min. 986 g/kg **metam-potassium:** min. 987 g/kg **Final specification at renewal is currently pending (please refer to Vol. 4 – Taminco).**

Table 70: Substance identity and information related to molecular and structural formula of MITC

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Methylisothiocyanatomethane
Other names (usual name, trade name, abbreviation)	MITC
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	209-132-5
EC name (if available and appropriate)	/
CAS number (if available)	556-61-6
Other identity code (if available)	Not available
Molecular formula	C_2H_3NS
Structural formula	CH3-N=C=S
SMILES notation (if available)	CN=C=S
Molecular weight or molecular weight range	73.11 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable: MITC has no stereoisomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable: MITC is not an UVCB substance
Degree of purity (%) (if relevant for the entry in Annex VI)	**Annex I - according to COMMISSION IMPLEMENTING REGULATION (EU) No 359/2012 of 25 April 2012:** max. 12 g/kg on dry weight basis (metam-sodium) max. 0.42 g/kg on dry weight basis (metam-potassium) **AIR 5:** Dry weight basis (calculated, modified equation to derive TC from TK values, refer to Vol. 4): max. 1.2 g/kg **(metam-sodium)** max. 0.5 g/kg **(metam-potassium)** **Final specification at renewal is currently pending (please refer to Vol. 4 – Taminco).**

2.11.1.2 Composition of the substance

Table 71: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Metam (incl. –sodium and – potassium) CAS numbers: 144-54-7 Variant 1: 137-42-8 Variant 2: 137-41-7 Note Metam-potassium (variant 2) self-classified in the C&L inventory under the name «Potassium methyldithiocarbamate»	**Min. purity expressed on a dry weight basis** **Annex I:** **metam-sodium:** min. 96.6% w/w **metam-potassium:** min. 99.0% w/w **Renewal:** 98.6% w/w (metam-sodium) 98.7 % w/w (metam-potassium) **Final specification at renewal is currently pending (please refer to Vol. 4 – Taminco).**	Metam potassium: not yet listed in Annex VI Metam sodium listed: H302, H314, H317, H400, H410	H302, H311, H314, H317, H332, H400, H410

Table 72: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
isothiocyanatomethane (MITC), CAS No. 556-61-6, EC No. 209-132-5	**Min. purity expressed on a dry weight basis** **Annex I:** max. 12 g/kg on dry weight basis (metam-sodium) max. 0.42 g/kg on dry weight basis (metam-potassium) **Renewal:** max. 1.2 g/kg **(metam-sodium)** max. 0.5 g/kg **(metam-potassium)**	H301, H314, H317, H331, H400, H410	H290, H301, H312, H314, H317, H330, H372, H400, H410	**No,** since it is a demonstrated main metabolite in mammals
1,3-Dimethylthiourea (DMTU), CAS No. 534-13-4, EC No. 534-13-4		Not listed in Annex VI	Not classified	**No**

Table 73: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
None*/					

*Note RMS: in the TGAI, an additive is put, as described in the confidential information, which is not necessary from a functional point of view, but which is unlikely to impact the human health classification of metam.

Table 74: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Metam* MITC				all

*metam as a TGAI might be salts of potassium or of sodium. The counterion is not likely to affect the overall toxicological profile, thus both forms were tested and considered equivalent.

2.11.2 Proposed harmonised classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 75: Proposed harmonised classification and labelling according to the CLP criteria (**metam**) **sodium and potassium**)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	006-0013-00-8	Metam-sodium (ISO); Sodium methyl-dithiocarbamate	205-293-0	137-42-8	Acute Tox. 4* Skin Corr. 1B Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H314 H317 H400 H410	GHS05 GHS07 GHS09 Dgr	H302 H314 H317 H410	EUH031		
RMS proposal at renewal	006-0013-00-8	metam-sodium (ISO); sodium methyldithiocarbamate [1]; **Add** metam-potassium (ISO); potassium methyldithiocarbamate [2];	205-293-0 [1]; **Add** 205-292-5 [2].	137-42-8 [1]; **Add** 137-41-7 [2]	**Retain** **Skin Sens. 1 Aquatic Acute 1** **Aquatic Chronic 1** **Modify** **Acute Tox. 4** **Skin Corr. 1** **Add** **Met Corr. 1** **Carc. 2** **Muta. 2** **Repr. 2** **Acute Tox 4** **STOT SE 1** **STOT RE 1**	**Retain** **H317** **H400** **H410** **Modify** **H302** **H314** **Add** **H290** **H351** **H341** **H361d** **H332** **H370 (liver)** **H372 (liver)**	**Retain** GHS05 GHS07 GHS09 Dgr **Add** **GHS08**	**Retain** **H317** **H410** **Modify** **H302** **H314** **Add** **H290** **H351** **H341** **H361d** **H332** **H370 (liver)** **H372 (liver)**	**Retain** EUH031	**Add** **inhalation:** **ATE = 1.5 mg/L** **oral:** **ATE = 500 mg/kg/d** **M = 1** **M = 1**	

Resulting Annex VI entry if agreed by RAC and COM	006-0013-00-8	metam-sodium (ISO); sodium methyldithiocarbamate [1]; metam-potassium (ISO); potassium methyldithiocarbamate [2]	205-293-0 [1]; 205-292-5 [2]	137-42-8 [1]; 137-41-7 [2]	Met Corr. 1 Carc. 2 Muta. 2 Repr. 2 Acute Tox 4 Acute Tox 4 STOT SE 1 STOT RE 1 Skin Corr. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H290 H351 H341 H361d H332 H302 H370 (liver) H372 (liver) H314 H317 H400 H410	GHS05 GHS07 GHS08 GHS09 Dgr	H290 H351 H341 H361d H332 H302 H370 (liver) H372 (liver) H314 H317 H410	EUH031	inhalation: ATE = 1,5 mg/L oral: ATE = 500 mg/kg/d M = 1 M = 1	

(added or modified classifications indicated in **bold** for convenience)

Table 76: Proposed harmonised classification and labelling according to the CLP criteria (**metam sodium and potassium**)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	none										
RMS proposal at renewal	006-0013-00-8	metam-sodium (ISO); sodium methyldithiocarbamate [1]; metam-potassium (ISO); potassium methyldithiocarbamate [2];	205-293-0 [1]; 205-292-5 [2].	137-42-8 [1]; 137-41-7 [2]	**Add** **Met. Corr. 1** **Acute Tox. 4** **STOT SE1** **STOT-RE 1** **Muta. 2** **Carc. 2** **Repr. 2** **Retain** Acute Tox. 4 **Modify** Skin Corr. 1 Retain Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**Add** **H290** **H332** **H370** **H372** **H341** **H351** **H361d** **Retain** H302 H314 H317 H400 H410	Retain GHS05 GHS07 GHS09 Dgr **Add** **GHS08**	**Add** **H332** **H370** **H372** **H341** **H351** **H361d** **Retain** H302 H314 H317 H410	EUH031	**Add** **M:** **Acute: 1** **Chronic: 1**	
Resulting Annex VI entry if agreed by RAC and COM											

(added or modified classifications indicated in **bold** for convenience)

Table 77: Proposed harmonised classification and labelling according to the CLP criteria (**MITC** main metabolite and breakdown product)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	615-002-00-2	Methyl isothiocyanate	209-132-5	556-61-6	Acute Tox. 3 Acute Tox. 3 Skin Corr. 1 B Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H301 H314 H317 H400 H410	GHS05 GHS06 GHS09 Dgr	H331 H301 H314 H317 H410			
RMS proposal at renewal	615-002-00-2	Methyl isothiocyanate	209-132-5	556-61-6	**Add** **Met. Corr. 1** **Acute Tox. 4** **STOT RE 1** **Carc. 2** **Modify** **Acute Tox. 2** Skin Corr. 1 **Retain** Acute Tox. 3 Skin Sens 1 Aquatic Acute 1 Aquatic Chronic 1	**Add** **H290** **H312** **H372** **H351** **Modify** **H330** **Retain** H314 **Retain** H301 H317 H400 H410	Retain GHS05 GHS06 Add **GHS08** GHS09 Dgr	Add H301 **Modify** **H330** **Add** **H312** **Retain** H314 H317 Add **H372** **H351** H410	**Add** **EUH071**	**Add** **M:** **Acute: 100** **Chronic: 10**	
Resulting Annex VI entry if agreed by RAC and COM											

2.11.2.2 Additional hazard statements / labelling

Table 78: Reason for not proposing harmonised classification and status under CLH public consultation – Metam (sodium and potassium)

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Data conclusive but not sufficient for classification	Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking but harmonised classification proposed claimed on the harmonised classification proposed for skin corrosion/irritation. Met. Corr. 1, H290	Yes
Acute toxicity via oral route	Acute Tox. 4, H302	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Acute Tox. 4, H332	Yes
Skin corrosion/irritation	Skin Corr. 1, H314	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation	Skin Sens. 1, H317	Yes
Germ cell mutagenicity	Muta. 2, H341	Yes
Carcinogenicity	Carc. 2, H351	Yes
Reproductive toxicity	Repr. 2, H361d	Yes
Specific target organ toxicity-single exposure	STOT SE 1, H370	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Specific target organ toxicity-repeated exposure	STOT RE 1, H372	Yes
Aspiration hazard	Not relevant	No
Hazardous to the aquatic environment	Aquatic Acute 1, H400; M=1 Aquatic Chronic 1, H410; M=1	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

Table 79: Reason for not proposing harmonised classification and status under CLH public consultation - MITC

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data lacking	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	yes
Oxidising gases	Hazard class not applicable	yes
Gases under pressure	Hazard class not applicable	yes
Flammable liquids	Hazard class not applicable	Yes
Flammable solids	Data lacking	Yes
Self-reactive substances	Data lacking	Yes
Pyrophoric liquids	Hazard class not applicable	yes
Pyrophoric solids	Data lacking	Yes
Self-heating substances	Data lacking	Yes
Substances which in contact with water emit flammable gases	Data lacking	Yes
Oxidising liquids	Hazard class not applicable	yes
Oxidising solids	Data lacking	Yes
Organic peroxides	Hazard class not applicable	yes
Corrosive to metals	Data lacking Harmonised classification proposed claimed on the harmonised classification proposed for skin corrosion/irritation.	Yes
Acute toxicity via oral route	Acute Tox.3, H301	Yes
Acute toxicity via dermal route	Acute Tox. 4, H312	Yes
Acute toxicity via inhalation route	Acute Tox. 2, H330	Yes
Skin corrosion/irritation	Skin corr. 1, H314	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation	Skin Sens. 1, H317	Yes/No
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Carcinogenicity	Carc. 2, H351	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	EUH071	Yes
Specific target organ toxicity-repeated exposure	STOT RE 1, H372	Yes
Aspiration hazard	Not relevant	No
Hazardous to the aquatic environment	Aquatic Acute 1, H400; M=100 Aquatic Chronic 1, H410; M=10	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

2.11.3 History of the previous classification and labelling

Harmonised classification (CLP00) exists at ECHA:

Index Number:	006-013-00-8
EC: European Community number	205-293-0
CAS Number:	137-42-8
International Chemical Identification	metam-sodium (ISO) sodium methyldithiocarbamate

2.11.4 Identified uses

Plant protection product (nematicide, fungicide, herbicide, insecticide) under Reg (EC) no 1107/2009.

2.11.5 Data sources

Plant protection product (nematicide, fungicide, herbicide, insecticide) evaluated under AIR-5, under Reg (EC) no 1107/2009.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

An exceedance was found of MITC in one use of metam, in greenhouse and walk-in tunnels (peppers), where the a.s. was applied at 306 kg/ha metam sodium, corresponding with 173.2 kg/ha MITC and 7.04 kg/ha DMTU in the Piacenza-scenario : 0.175 µg/L.

2.12.1 STEP 1: Exclusion of degradation products of no concern

MITC is no substance of no concern.

2.12.2 STEP 2: Quantification of potential groundwater contamination

MITC: 0.175 µg/L

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

MITC is the ultimate metabolite of metam, and is thus by definition biologically active.
Consequently, the metabolite is environmentally relevant, and should not exceed the trigger level of 0.1 µg/L in groundwater.

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

MITC is not genotoxic.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

MITC is toxic by inhalation Acute Tox 2, H330), and may possibly be classified as a Carc Cat.2 (to be confirmed during the peer review). Takin into account the acute toxicity, the metabolite MITC is considered relevant and should not exceed the trigger level of 0.1 µg/L in groundwater.
All metabolites not passing stage 3 of step 3 are considered relevant and are in principle not subject to an exposure and risk assessment.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

MITC is relevant, and the application leading to an exceedance of 0.1 µg/L in groundwater should not be part of the intended use.

2.12.5 STEP 5: Refined risk assessment

MITC is relevant, and the application leading to an exceedance of 0.1 µg/L in groundwater should not be part of the intended use.

2.12.6 Overall conclusion

MITC is relevant, and the application leading to an exceedance of 0.1 µg/L in groundwater should not be part of the intended use.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.13.1 Identity and physical chemical properties

Not applicable. Neither metam (including its variants sodium and potassium), nor its metabolites MITC or DMTU contain a stereogenic center.

2.13.2 Methods of analysis

Not applicable. Neither metam (including its variants sodium and potassium), nor its metabolites MITC or DMTU contain a stereogenic center.

2.13.3 Mammalian toxicity

Not applicable. Neither metam (including its variants sodium and potassium), nor its metabolites MITC or DMTU contain a stereogenic center.

2.13.4 Operator, Worker, Bystander and Resident exposure

Not applicable. Neither metam (including its variants sodium and potassium), nor its metabolites MITC or DMTU contain a stereogenic center.

2.13.5 Residues and Consumer risk assessment

Not applicable. Neither metam, nor its metabolites MITC or DMTU contain a stereogenic center.

2.13.6 Environmental fate

2.13.7 Ecotoxicology

Not applicable. Neither metam (including its variants sodium and potassium), nor its metabolites MITC or DMTU contain a stereogenic center.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin:

(1) methyl isothiocyanate (MITC)
(2) N,N'-dimethylthiourea (DMTU)

Food of animal origin: inconclusive

Soil:

Groundwater:

Surface water:

Sediment:

Air:

2.14.2 Definition of residues for monitoring

Food of plant origin: methyl isothiocyanate (MITC) (resulting from the use of dazomet or metam)

Food of animal origin: inconclusive

Soil: methyl isothiocyanate (MITC)

Groundwater: methyl isothiocyanate (MITC)

Surface water: methyl isothiocyanate (MITC)

Sediment: methyl isothiocyanate (MITC)

Air: methyl isothiocyanate (MITC)

Level 3

METAM

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		**A.s.:** Metam sodium and -potassium, having as the ultimate metabolite MITC (actual substance responsible for the biological action) **Formulations :** Metam Sodium 51% SL and Metam Sodium 510 SL
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted		X	*[If no go to ii immediately below]*
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			Please refer to section 3.1.4 where further information is required.
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.	X		Please refer to section 3.1.4 where further information is required.
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on	X		

	feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Representative uses are already authorized on national level and have been evaluated according to uniform principles. See level 2.3.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		Note: For the relevant metabolite/impurity DMTU, which may occur in crops, the information available is insufficient to derive toxicological reference values. However, toxicological reference values of MITC were taken as surrogate worst-case values for the consumer dietary risk assessment of DMTU.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.		X	New 5 batch analyses based on large scale production have been submitted by both applicants for the purpose of renewal. Nevertheless, the final specification at renewal, especially the specification derived on a dry weight basis) is pending on further information (for details please refer to the respective Volume 4 of Taminco BV and Lainco SA).

				Further information is required to support the specification at renewal of metam (please refer to section 3.1.4.1). An update of the initial reference specification and EU agreed levels appears to be not required from a tox/ecotox perspective. It is however questioned if a revision of the EU agreed levels and an update of the reference specification should not occur and would not be more appropriate in this case even not fully justify from a (eco)tox. perspective (see details in Vol.4 – Taminco and Vol.4 – Lainco).
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted	X		
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.		X	The metam sodium or metam potassium content in technical concentrate is determined by the CIPAC method (CS_2 evolution method). HPLC-UV methods are available for the determination of the relevant impurities DMTU and MITC. However, a data gap is identified for Lainco SA regarding the validation of the method to determine MITC: please refer to Level 2.5.1.1. Validated methods for determining impurities are also available (see respective Vol. 4 for further discussion/information) .
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	Please refer to details and data gaps identified in Level 2.5.2.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				

		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		**Metam** (both sodium and potassium salts): ADI = 0.001 mg/kg bw/d ARfD = 0.1 mg/kg bw/d AOEL = 0.001 mg/kg bw/d AAOEL = 0.001 mg/kg bw/d **MITC**: ADI = 0.004 mg/kg bw/d ARfD = 0.03 mg/kg bw/d AOEL = 0.004 mg/kg bw/d AAOEL* = 0.004 mg/kg bw/d *based on the inhalation rabbit developmental study (maternal NOAEL =1.1 mg/kg bw/d, safety factor 300 = 100 × 3). The additional safety factor of 3 takes into account the severity of the effect at LOAEL (lethality).
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	**Metam** is proposed to be classified for mutagenicity in cat.2. The justification is provided in the CLH part in this volume 1. RMS considers that the endpoint observed is not severe enough to trigger a classification as Cat 1B. **MITC** is proposed to be not classified for mutagenicity. The justification is provided in the CLH part in this volume 1. The issue should be discussed in an expert consultation.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**			**Metam** is proposed to be classified for carcinogenicity in cat.2. The justification is provided in the CLH part in this volume 1. RMS considers that the endpoint observed is not severe enough to trigger a classification as Cat 1B. **MITC** is proposed to be classified for carcinogenicity in cat.2. The justification is provided in the CLH part in this volume 1. RMS considers that the endpoint observed is not severe enough to trigger a classification as Cat 1B. The issue should be discussed in an expert consultation.
ii)	Linked to above classification proposal.			Not of application, since no C&L in cat. 1B is proposed.

	It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			**Metam** is proposed to be classified for reprotoxicity (development) in cat.2. The justification is provided in the CLH part in this volume 1. RMS considers that the endpoint observed is not severe enough to trigger a classification as Cat 1B. **MITC** is proposed to be not classified for reprotoxicity. The justification is provided in the CLH part in this volume 1. The issue should be discussed in an expert consultation.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not of application, since no C&L in cat. 1B is proposed.
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**			Interim criteria for ED not relevant anymore. The a.s. metam and its metabolite MITC were evaluated according the guidance of EFSA/ECHA for endocrine endpoints. **Conclusion:** to date (July 2021), the planned additional studies mentioned in 2.10 (**OECD TG 458**, **OECD TG 456**, **OPPTS 890.1200** and, potentially, **OECD TG 441**) are not available for toxicology, and the planned additional studies mentioned hereabove (**OECD TG 229** and **OECD TG 231**) are not available for ecotoxicology. As a consequence, a complete picture of the ED landscape may not be obtained yet, hence a definitive conclusion on ED may not be drawn.

				See also section 3.1.4 : list of studies to be generated, still ongoing or available but not peer reviewed.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**			Interim criteria for ED not relevant anymore, see above.
iii)	Linked to either i) or ii) immediately above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not of application, further studies awaited as described above.
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	<u>*Water = no*</u> *DT_{50} water for MITC = 10.5 days (worst case) < 2 months.* <u>*Soil = no*</u> *Field soil degradation studies carried out in EU show that DT_{50} values of MITC are < 6 months (worst-case DT_{50} =2.03 days)* <u>*Sediment = no*</u> *Water/sediment studies show that DT_{50} values of MITC are < 6 months (DT_{50} whole system = 15 days (worst-case for aerated systems), DT_{50} water = 10.5 days (worst-case in aerated systems).* ➔ *Based on available data, MITC is not considered as Persistent regarding the POP criteria* *log P_{OW} MITC at 20°C: 1.05 (pH 7.5, 97.0 %) (below trigger value of 3), no study was requested to determine the BCF.* *MITC is not considered as potentially bioaccumulative regarding the POP criteria* <u>*Air*</u> *$DT_{50 >}$ 2 days*

				MITC is considered as potential for long-range environmental transport
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Water = no DT_{50} water MITC = 10.5 days (worst case) < 2 months. Sediment = no Water/sediment study shows that DT_{50} values of MITC are < 6 months (DT_{50} whole system = 15 days (worst-case), DT_{50} water =10.5 days (worst-case)). Soil = no ➔ Field soil degradation studies carried out in EU show that DT_{50} values of MITC are < 120 days (worst-case DT_{50} MITC = 2.03 days) ➔ Based on available data, MITC is not considered as Persistent regarding the PBT criteria Log Pow for MITC is 1.05 (below trigger value of 3), no study was requested to determine the BCF. MITC is not considered as potentially bioaccumulative regarding the POP criteria The lowest RAC for all aquatic organisms is < 0.01 mg/L (RAC = 0.531 µg a.s./L, based on CA8.2.1/10: acute fish study with Oncorhynchus mykiss). MITC is not considered toxic regarding the PBT criteria.
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Water = no DT_{50} water = 10.5 days (worst case) < 2 months. Sediment = no Water/sediment studies show that DT_{50} values of MITC are < 180 days (DT_{50} whole system = 15 days (worst-case), DT_{50} water = 10.5 days). Soil = no Field soil degradation studies carried out in EU show that DT_{50} values of MITC are < 180 days (worst-case DT_{50} =2.03 days). ➔ Based on available data, MITC is not considered as Persistent regarding the vPvB criteria Log Pow for MITC is 1.05 (below trigger value of 3), no study was requested to determine the BCF. MITC is not considered as potentially bioaccumulative regarding the POP criteria
Ecotoxicology				
		Yes	No	

i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.		X	For all proposed uses, the risk to birds and mammals was acceptable at screening step or Tier 1 (exposure only possible through inhalation or following redeposition after volatilisation). For the proposed uses in permanent greenhouses by applicant Eastman (Taminco), the risk to aquatic organisms was acceptable based on reliable exposure calculations. However, for the proposed uses indoor (permanent greenhouses and walk-in tunnels) and outdoor by the applicant Lainco, the risk to aquatic organisms is unconclusive since the exposure calculations are underestimating the risk (most relevant route of exposure by volatilisation was not integrated in the calculations). RMS considers this as an issue not finalised and further refinement is needed. For all proposed uses, the risk to bees was acceptable at Tier 1 (exposure only possible through inhalation or following redeposition after volatilisation). For non-target arthropods, earthworms and other soil meso- and macrofauna, the risk is acceptable based on field studies. A low risk to soil micro-organisms was demonstrated based on higher tier laboratory data (using aged soil, sampled from a metam-sodium-treated field). The risk to non-target terrestrial plants was acceptable based on Tier 1 studies, as was the risk to biological methods for sewage treatment. See Section B.9.1 to B.9.14 of Volume 3(PPP) for further details.
ii	It is considered that, the substance **SHOULD BE identified as having endocrine disrupting properties** that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.			For both the T-modality and the EAS-modalities, endocrine adversity and activity have not been sufficiently investigated. The notifier agreed on performing additional studies to complete the dataset. These studies have however not yet been submitted. As a consequence, a complete picture of the ED landscape may not be obtained yet, hence a definitive conclusion on ED may not be drawn.
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.		X	Non-target organisms are potentially exposed to the active substance metam when used according to the proposed uses.
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or	X		Due the method of application (to bare soil, through soil injection or drip irrigation, followed by a period of 3 to 6 weeks coverage with TIF), direct oral or contact exposure to bees in the field or greenhouse during application is considered to be negligible. By the time flowering weeds or crops will be present in-field, remaining MITC residues in the soil will be negligible since seeds will not germinate if significant residues are still present. Further, MITC

	— has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			is not systemic, and therefore oral exposure to bees at later stages of the crop growth can also be considered negligible. In addition to the above, residues in subsequent crops (and weeds) will be negligible, as confirmed by crop residue studies (See Volume 3 (CA) Section B.7.2). Furthermore, metam is intended to be applied at a maximum of once every third year on the same field, and given the rapid breakdown of metam and MITC, no chronic exposure to adult bees or bee larvae is expected in- or off-field. Off-field exposure to MITC vapours through inhalation might be possible. However, a Tier 1 risk assessment based on measured air concentrations and laboratory inhalation toxicity studies indicated an acceptable risk. Off-field exposure (both oral and contact) through redeposition after volatilization (off-field) might also be possible. However, estimated exposure levels (assuming that from the maximum measured concentrations in air, all the MITC present in one m^3 is deposited on a 1 m^2 off-field area of soil or plants) are very low (between, 0.0009 and 0.006% of the in-field application rate). Therefore, the risk to bees from this exposure route is considered low.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X	X	Residue definition(s) for risk assessment and enforcement could be established for commodities of plant origin (see 2.7.3.1). However, a residue definition for commodities of animal origin could not be concluded on (see 2.7.3.2).
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X	X	For details on the groundwater assessment, please refer to section 2.8.6. For all uses the PEC_{GW} values for MITC and the impurity DMTU are below the trigger of 0.1 µg/L. Except for the use in pepper (Lainco SA) PEC_{GW} values for MITC were below the 0.1 µg/L in all scenarios and timings except the October PIACENZA scenario, where the PEC_{GW} was 0.174959 µg/L.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution			Formally, metam/MITC are to be considered CfS. It is to be discussed in an expert consultation. Pending the final evaluation on ED and CMR, the issue cannot be concluded, but at least the ADI/AOEL value metam meets the criterion, and 2 conditions of PBT are fulfilled. Even if overall, both a.s. escape the rest of the criteria, the past experience learns that EU COM did classify similar a.s. on the basis of the ADI alone. The general issue is anyway risk management matter.

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Conclusion on final specification at renewal (Taminco BV) is pending on: - Further information from Taminco BV regarding determination of some impurities (see Vol.4 – Taminco) which can have an influence on the way to calculate the dry weight content of active substance and impurities based on the TK values; - Further information from Taminco BV regarding final proposed level of MITC (see Vol. 4 – Taminco)		X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
CLH – proposal for classification as Corr. To metals cat. 1 – H290 (metam - MITC): Classification is claimed based on experience. However, Taminco BV is requested to provide some experimental evidences of incompatibilities to metals observed on sites, if any, in full support of the statement.		X		

3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
Analytical method to determine the relevant impurity MITC in TK and PPP (Lainco SA): Method should be further validated at a lower level appropriate to batches and specification.	All	X		
For study "*Methyl Isothiocyanate (MITC): An Acute Vapor Exposure Toxicity Study with the Honey Bee*" (2016, report 703H-101), the notifier (Taminco BV) is requested to provide further clarifications on the high variability observed in the analysed exposure concentrations of MITC.	All	X		
Lainco SA is requested to provide validation Renolab study 12054-07 R on carrot.	carrot			X
Monitoring method in plants (Tamino BV and Lainco SA): Further quantitative validation data on confirmative ions for MITC are required.	All	X (Taminco BV)		X (Lainco SA: study available but an amended report including validation data on confirmative ions needs to be provided)
Monitoring method in soil (Lainco SA):	All			X (Lainco SA: study available and

Further quantitative validation data on confirmative ions for MITC.				considered, an amended report including validation data on confirmative ions needs to be provided)
Monitoring method in body fluids and tissues (Lainco SA): A validated method for determination of MITC in liver should be provided.	All	X		
3.1.4.6 Toxicology and metabolism				
(Studies on ED). · **In vitro androgen transactivation assay (OECD TG 458).**	Study needed to conclude on ED potential		X (Q1 2021)	
(Studies on ED). · ***In vivo* Hershberger assay (OECD TG 441 (only if OECD TG 458 is negative).**	Study needed to conclude on ED potential		X (Q1 2021)	
(Studies on ED). ***In vitro* H295R steroidogenesis assay (OECD TG 456)**	Study needed to conclude on ED potential		X (Q1 2021)	
(Studies on ED). ***In vitro* aromatase assay (human recombinant) (OPPTS 890.1200)**	Study needed to conclude on ED potential		X (Q1 2021)	
(Product Metam Na 510 SL – Taminco) Notifier Taminco announced the conduct of two further exposure studies, possibly accompanied by their corresponding modelling studies. These studies were not available at the moment of finalization; however, a conclusion has been	Supplementary studies	X		

drawn based on the available studies (3 on exposure and 3 on modelling).				
(Product Metam Sodium 51 SL – Lainco) Notifier Lainco announced that new data would be generated with the use of TIF to cover the treated area, and that the first results would be available in 2020. However, these studies were not available at the moment of finalization. Nevertheless, a conclusion has been drawn based on the available study.	Supplementary studies	X		
3.1.4.7 Residue data				
At least 3 additional GAP-compliant supervised residue trials on potatoes (conducted in SEU)	Potatoes		X (Q1 2021)	
At least 1 additional GAP-compliant supervised residue trial on carrots (conducted in SEU)	Carrots		X (Q2 2021)	
At least 6 additional GAP-compliant supervised residue trials on onions (conducted in SEU)	Onions		X (2021)	
At least 4 additional GAP-compliant supervised residue trials on pepper (conducted indoor) to confirm the <LOQ residue situation	Pepper	X		
Information/data that could reasonably justify the omission of the available supervised residue trials on potatoes, carrots and onions in which (relatively high) quantifiable levels of MITC and DMTU were observed.	Potato, carrots, onions	X		
Information/data to exclude potential residues in honey and other apiculture products	Pepper	X		
3.1.4.8 Environmental fate and behaviour				
(Product Metam Sodium 51 SL – Lainco)	Potato, carrot, onions	X		

PEC_{GW} recalculation for MITC taking into account the diffusion of compounds in the gas phase.				
(Product Metam Sodium 51 SL – Lainco) PEC_{GW} recalculation for MITC taking into account the diffusion of compounds in the gas phase and an incorporation of 20 cm.	Pepper	X		
(Product Metam Sodium 51 SL – Lainco) PEC_{GW} recalculation for DMTU using the validated endpoint and an incorporation of 20 cm.	Potato, carrot, onion, pepper	X		
(Product Metam Sodium 51 SL – Lainco) Data on volatilisation and subsequent deposition of the compound MITC following the application of the product Metam Sodium 51 SL according to the GAP applied for (field and under protection).	Potato, carrot, onion, pepper	X		
(Product Metam Sodium 51 SL – Lainco) PEC_{SW} and PEC_{SED} recalculation using data on volatilisation and subsequent deposition.	Potato, carrot, onion, pepper	X		
(Product Metam Na 510 SL – Taminco) Two additional exposure studies where measurements of redeposition of MITC to surface water are included are on-going. These studies were not available at the moment of finalization; however, a conclusion has been drawn based on the available studies.	Supplementary studies		X (Q3 2021 - Q4 2021)	
3.1.4.9 Ecotoxicology				
(Studies on ED). · **Fish short-term reproduction assay (OECD TG 229).**	Study needed to conclude on ED potential		X (Q1 2021)	

(Studies on ED). · **Apmhibian metamorphosis assay (OECD TG 231).**	Study needed to conclude on ED potential		X (Q1 2021)	

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)
1. Conclusion on final specification is pending on further information	All representative uses
2. Consumer dietary risk assessment (due to data gaps and uncertainties on magnitude of residues in primary crops)	Potatoes, carrots, onions, peppers
3. Groundwater risk assessment (due to a lack of information that should be considered in the calculations)	Potatoes, carrots, onions, peppers
4. Surface water risk assessment (due to a lack of information that should be considered in the calculations)	Potatoes, carrots, onions, peppers
5. Aquatic risk assessment (pending on surface water risk assessment in fate section)	Potatoes, carrots, onions, peppers

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
5. Exceedance of groundwater exposure by metabolite MITC	MITC is the ultimate metabolite of metam, and is thus by definition biologically active. Consequently, the metabolite is environmentally relevant, and should not exceed the trigger level of 0.1 µg/L in groundwater. In a worse-case scenario, MITC gw level is predicted to be 0.175 µg/L (greenhouse pepper, 306 kg/ha metam/ha), and this use is thus unacceptable.

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Lettuce	Baby leaf crops	Potatoes	Carrots	Onions	Peppers	Ornamental crops
Operator risk	Risk identified							
	Assessment not finalised							
Worker risk	Risk identified							
	Assessment not finalised							
Bystander risk	Risk identified							
	Assessment not finalised							
Consumer risk	Risk identified							
	Assessment not finalised			X^{2}	X^{2}	X^{2}	X^{2}	
Risk to wild non target terrestrial vertebrates	Risk identified							
	Assessment not finalised							
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified							
	Assessment not finalised							
Risk to aquatic organisms	Risk identified							
	Assessment not finalised			X^{5}	X^{5}	X^{5}	X^{5}	
Groundwater exposure active substance	Legal parametric value breached							
	Assessment not finalised							
Groundwater exposure metabolites	Legal parametric value breached						X^{3}	

	Parametric value of 10µg/L[(a)] breached							
	Assessment not finalised							
Comments/Remarks								

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
Consumer dietary exposure to residues	RMS recommends a consultation of experts on the representativeness and reliability of the available supervised residue trials, which showed unexplained variable residue findings; see 2.7.4. RMS proposes in particular to discuss whether it is justified to omit the quantifiable residue findings observed in older trials and how the reliability of certain results needs to be judged taking into account uncertainties on storage stability of residues in some matrices (e.g. for pepper); see 2.7.1.
CMR evaluation and classification	RMS recommends a consultation of experts on the suggested classification of metam as Muta 2, Carc 2 and Repro 2, and on the suggested classification of MITC as Carc 2.
ED properties	RMS identified data gaps in the ED assessment of metam. Therefore, notifier planned to conduct these studies which should be evaluated in order to finalise the issue of ED properties of this a.s..
PEC_{GW} and PEC_{SW} calculation	RMS and Co-RMS recommend a consultation of experts on the approach followed by Taminco BV to calculate PEC_{GW} and PEC_{SW} values.
Aquatic risk assessment	For the proposed uses indoor (permanent greenhouses and walk-in tunnels) and outdoor by the applicant Lainco, the risk to aquatic organisms is unconclusive since the exposure calculations are underestimating the risk (most relevant route of exposure by volatilization was not integrated in the calculations). Expert consultation in ecotoxicology is pending on the comments and discussions in the fate section regarding the predicted environmental concentrations in surface water for a soil fumigant.

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
None received		

3.2 PROPOSED DECISION

[redacted]

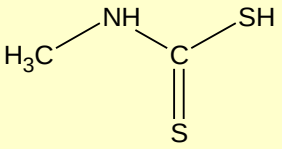
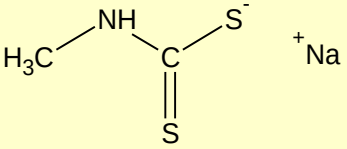
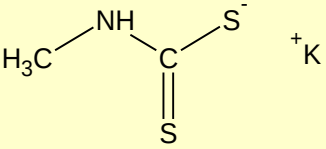
3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

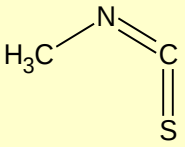
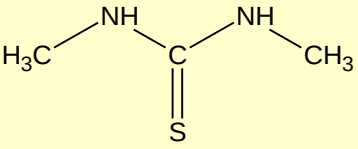
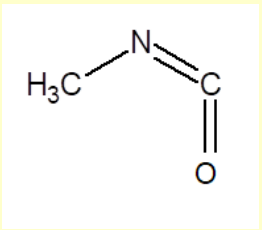
3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

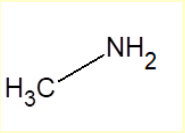
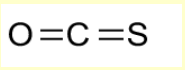
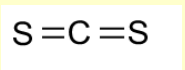
Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	

3.4 APPENDICES

3.4.1 LIST OF METABOLITES OF METAM

Code Number (Synonyms)	IUPAC name /CAS. No. /SMILES notation /InChiKey)	Structural formula	Compound found in:
Metam	IUPAC name: Methyldithiocarbamic acid CAS No.: 144-54-7 SMILES: N(C(=S)S)C InChiKey: HYVVJDQGXFXBRZ-UHFFFAOYSA-N		**Mammals**: Plasma (0.044-0.33 µg/g at 168 h; peak: 1.84 µg/mL after 30 min)) Tissues (1.17-2.1% of TRR) (organs with highest residues: liver, kidney, lung, adrenals, thyroid) Urine (37-58% of TRR) Expired air (50% of TRR) **Soil** as applied
Metam-sodium	IUPAC name: Sodium N-methyldithiocarbamate CAS No.: 137-42-8 SMILES: C(=S)(NC)[S-].[Na+] InChiKey: AFCCDDWKHLHPDF-UHFFFAOYSA-M		See Metam
Metam-potassium	IUPAC name: Potassium N-methyldithiocarbamate CAS No.: 137-41-7 SMILES: C(=S)(NC)[S-].[K+] InChiKey: DQRQIQZHRCRSDB-UHFFFAOYSA-M		See Metam

Code Number (Synonyms)	**IUPAC name /CAS. No. /SMILES notation /InChiKey)**	**Structural formula**	**Compound found in:**
MITC	IUPAC name: Methyl isothiocyanate CAS No.: 556-61-6 SMILES: CN=C=S InChiKey: LGDSHSYDSCRFAB-UHFFFAOYSA-N		**Mammals**: Plasma (0.013-0.14 µg/g at 168 h; peak: 1.6 µg/mL after 30 min) Tissues (1.71-2.29% of TRR) (organs with highest residues: liver, kidney, lung, adrenals, thyroid) Urine (84-87% of TRR) Expired air (8-17% of TRR) **Soil** (aerobic, max 82.9% AR) **Water/sediment** systems (potentially by drainage and/or volatilisation/redeposition) Field studies ongoing **Air** (main degradation product during soil fumigation)
DMTU	IUPAC name: 1,3-Dimethylthiourea CAS No.: 61805-96-7 SMILES: CNC(=S)NC InChiKey: VLCDUOXHFNUCKK-UHFFFAOYSA-N		**Soil** (as impurity, max 23 g/kg metam-sodium on a dry weight basis)
MIC	IUPAC name: methylisocyanate CAS No.: 624-83-9 SMILES: CN=C=O InChiKey: HAMGRBXTJNITHG-UHFFFAOYSA-N		**Air** (photolysis)

Code Number (Synonyms)	IUPAC name /CAS. No. /SMILES notation /InChiKey)	Structural formula	Compound found in:
CH_3-NH_2	IUPAC name: Methanamine Methylamine CAS No.: 74-89-5 SMILES: CN InChiKey: BAVYZALUXZFZLV-UHFFFAOYSA-N		**Mammals**: expired air **Air**
H_2S	IUPAC name: sulfane Hydrogen sulfide CAS No.: 7783-06-4 SMILES: S InChiKey: RWSOTUBLDIXVET-UHFFFAOYSA-N	H_2S	**Mammals**: expired air **Air**
COS	IUPAC name: Carbon oxide sulfide Carbonyl sulfide CAS No.: 463-58-1 SMILES: C(=O)=S InChiKey: JJWKPURADFRFRB-UHFFFAOYSA-N		**Mammals**: expired air **Air**
CS_2	IUPAC name: Carbon disulfide CAS No.: 75-15-0 SMILES: C(=S)=S InChiKey: QGJOPFRUJISHPQ-UHFFFAOYSA-N		**Mammals**: expired air **Air**

Remark RMS: relevant air degradates MIC, methylamine, H_2S, COS, CS_2, not mentioned in doc N3 of the notifiers.

3.4.2 LIST OF GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

See *"Commission Communication in the framework of the implementation of Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market"* (2013/C 95/01)

See *"Commission communication in the framework of the implementation of Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market"* (2013/C 95/02)

Section identity, physical chemical and analytical methods

Section physical chemical properties

Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No. 1107/2009, SANCO/10597/2003, rev.10.1

Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO/3030/99 rev.5.

WHO/FAO. 2016. Manual on development and use of FAO and WHO specifications for pesticides. Third revision of the first edition. Rome, 2016

Section analytical methods

Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO/3030/99 rev.5.

Guidance document on pesticides residue analytical methods, SANCO/825/00 rev. 8.1

Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4.

Technical Guideline on the evaluation of Extraction Efficiency of Residues Analytical Methods SANTE 2017/10632 Rev. 3.

Section Data on application and efficacy

EPPO Guideline PP 1/213 (4) Resistance risk analysis (Bulletin OEPP/EPPO Bulletin (2015) 45 (3), 371–38).

Guidelines for the Preparation of a Biological Assessment Dossier, 7600/VI/95 rev. 6 (as amended by PSD), v1.03 04/11/2010.

Section Toxicology

Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC; Sanco/221/2000 –rev.10- final 25 February 2003

Guidance on Dermal Absorption, EFSA Panel on Plant Protection Products and their Residues (PPR) -

European Food Safety Authority (EFSA), EFSA Journal 2017;15(6):4873.

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009; European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC), EFSA Journal 2018; 16(6) e05311. https://doi.org/10.2903/j.efsa.2018.5311

Section Residue and consumer risk assessment

EC, 2013. Working document on the nature of pesticide residues in fish (European Commission, 31 January 2013 – SANCO/11187/2013 Rev. 3)

EC, 2017. European Commission – Guidance Document: Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95-rev. 10.3. 13 June 2017

OECD, 2013. OECD guidance document No 73 on residue in livestock (Sept. 2013). [Series on Pesticides No. 73; ENV/JM/MONO(2013)8]

Section fate and behaviour in environment

Section ecotoxicology

European Food Safety Authority, 2009; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA, EFSA Journal 2009; 7(12):1438.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290.

Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002, rev 2 (final) 17 October 2002.

Candolfi *et al*. (2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing), Wageningen, NL, 21-23 March 2000, SETAC Europe. SETAC publication, August 2001.

3.4.3 REFERENCE LISTS

Section identity, physical chemical and analytical methods

Belgium, 2007/2008. Draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2007, updated June 2008. Available online: www.efsa.europa.eu

Belgium, 2010b. Revised draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2010. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2011. Conclusion on the peer review of the pesticide risk assessment of the active substance metam. EFSA Journal 2011;9(9):2334, 97 pp. https://doi.org/10.2903/j.efsa.2011.2334

Section data on application and efficacy

Section toxicology

Belgium, 2007/2008. Draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2007, updated June 2008. Available online: www.efsa.europa.eu

Belgium, 2010b. Revised draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2010. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2011. Conclusion on the peer review of the pesticide risk assessment of the active substance metam. EFSA Journal 2011;9(9):2334, 97 pp. https://doi.org/10.2903/j.efsa.2011.2334

Following references from foreign authorities **outside the EU** may be cited and referred to as regards the evaluation of MITC-releasing active substances:

Thongsinthusak T *et al*, 2002, The Department of Pesticide Regulation's document: Evaluation of methyl isothiocyanate (MITC) as a toxic air contaminant. Regulation's draft report titled "Evaluation of Methyl Isothiocyanate (MITC) as a Toxic Air Contaminant"). Part B, Exposure Assessment
Scientific Review Panel, California, based on the Panel's review of the Department of Pesticide

Rubin A *et al*, 2002, The Department of Pesticide Regulation's document: Evaluation of methyl isothiocyanate (MITC) as a toxic air contaminant. Regulation's draft report titled "Evaluation of Methyl Isothiocyanate (MITC) as a Toxic Air Contaminant"). Part C, Health Assessment
Scientific Review Panel, California, based on the Panel's review of the Department of Pesticide

Rubin A, *et al*, 2003, Risk characterization document, Methyl Isothiocyanate (MITC) Following the Agricultural Use of Metam Sodium, Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency.

NRA Special Review of Metham Sodium, Dazomet and Methylisothiocyanate (MITC), Volume II, 1997, NRA Special Review Series 97, Chemical Review Section, National Registration Authority, Australia.
(provides the summary reports of the assessment of toxicological data for metham, dazomet and MITC, including a summary of comparative toxicology of the three compounds. It also contains the occupational health and safety (OH&S) risk assessment of metham (soil fumigant use) dazomet and MITC and provides recommendations for use of dazomet and soil fumigant use of metham. This volume also includes an OH&S risk assessment of root inhibitor use of metham and recommendations for use of metham as a root

inhibitor.)

NRA Special Review of Metham Sodium, Dazomet and Methylisothiocyanate (MITC), Volume III, 1997, NRA Special Review Series 97, Chemical Review Section, National Registration Authority, Australia. (contains the full reports of the toxicological assessments for Metham-Sodium, Dazomet and MITC).

Section residue and consumer risk assessment

Belgium, 2007/2008. Draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2007, updated June 2008. Available online: www.efsa.europa.eu

Belgium, 2009. Additional report to the draft assessment report on the active substance dazomet prepared by the rapporteur Member State Belgium in the framework of Commission Regulation (EC) No 33/2008, December 2009. Available online: www.efsa.europa.eu

Belgium, 2010b. Revised draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2010. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance metam. EFSA Scientific Report (2008) 203.

EFSA (European Food Safety Authority), 2011. Conclusion on the peer review of the pesticide risk assessment of the active substance metam. EFSA Journal 2011;9(9):2334, 97 pp. https://doi.org/10.2903/j.efsa.2011.2334

EFSA (European Food Safety Authority), 2019a. Reasoned Opinion on the review of the existing maximum residue levels for metam according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2019;17(1):5561, 76 pp. https://doi.org/10.2903/j.efsa.2019.5561

EFSA (European Food Safety Authority), 2019b. Reasoned opinion on the review of the existing maximum residue levels for dazomet according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2019;17(1):5562, 100 pp. https://doi.org/10.2903/j.efsa.2019.5562

France, 2017. Evaluation report prepared under Article 12 of Regulation (EC) No 396/2005. Authorised uses to be considered for the review of the existing EU MRLs for metam, June 2017. Available online: www.efsa.europa.eu

Section fate and behaviour in environment

Section ecotoxicology

Belgium, 2007/2008. Draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2007, updated June 2008. Available online: www.efsa.europa.eu

Belgium, 2010b. Revised draft assessment report on the active substance metam prepared by the rapporteur Member State Belgium in the framework of Council Directive 91/414/EEC, August 2010. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2011. Conclusion on the peer review of the pesticide risk assessment of the active substance metam. EFSA Journal 2011;9(9):2334, 97 pp. https://doi.org/10.2903/j.efsa.2011.2334