Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4’-(o-phenylene)bis(3-thioallophanate)

EC Number: 245-740-7
CAS Number: 23564-05-8

CLH-O-0000001412-86-281/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as ‘RAC evaluation’. Only the RAC text reflects the view of RAC.

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate)

EC Number: **245-740-7**

CAS Number: **23564-05-8**

Index Number: **006-069-00-3**

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Version number: 4 **Date: 28 March 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON THIOPHANATE-METHYL (ISO); DIMETHYL (1,2-PHENYLENEDICARBAMOTHIOYL)BISCARBAMATE; DIMETHYL 4,4′-(O-PHENYLENE)BIS(3-THIOALLOPHANATE)

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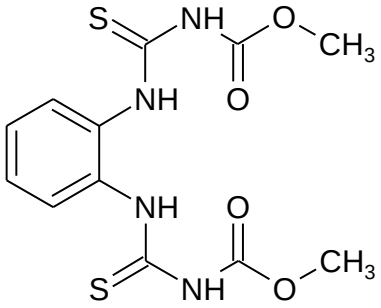
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: 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)	IUPAC: dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate CA: Carbamic acid, N,N'-[1,2-phenylenebis(iminocarbonothioyl)]bis-, C,C'-dimethyl ester
Other names (usual name, trade name, abbreviation)	Topsin M
ISO common name (if available and appropriate)	Thiophanate-methyl
EC number (if available and appropriate)	245-740-7
EC name (if available and appropriate)	Thiophanate-methyl
CAS number (if available)	23564-05-8
Other identity code (if available)	262 (CIPAC)
Molecular formula	$C_{12}H_{14}N_4O_4S_2$
Structural formula	
SMILES notation (if available)	S=C(Nc1ccccc1NC(=S)NC(=O)OC)NC(=O)OC
Molecular weight or molecular weight range	342.40 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable; thiphanate-methyl does not consist of sterero-isomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant; thiophanate is not an UVCB substance
Degree of purity (%) (if relevant for the entry in Annex VI)	95%

1.2 Composition of the substance

Table 2: 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)
Thiophanate-methyl	Min. 95%	Acute Tox. 4* Skin Sens. 1 Muta. 2 Aquatic Acute 1 Aquatic Chronic 1	Acute Tox. 4 Skin Sens. 1 Muta. 2 Aquatic Acute 1 Aquatic Chronic 1

Table 3: 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
2,3-diaminophenazine (DAP) CAS-No.: 655-86-7	max. 0.5 ppm	-	23 out of 30 notifiers has the following proposal: Acute Tox. 4, H302 Skin Irrit. 2, H315 Eye Irrit. 2, H319 STOT SE 3., H335 (not provided) GHS07 Wng One further notifier proposes as above with the following addition: Acute Tox. 4, H312 Acute Tox. 4, H332 STOT SE 3, H335 (Lungs) One notifier has only H302 GHS07 Wng	No
2-amino-3-hydroxyphenazine (HAP) CAS-No.: 4569-77-1	max. 0.5 ppm	-	-	No
Carbendazim CAS-No.: 10605-21-7	max. 0.09%	Muta. 1B, H340 Repr. 1B, H360FD Aquatic Acute 1, H400 Aquatic Chronic 1, H410	There are 25 aggregated notifications comprising approximately 500 notifiers. Most are proposal are identical to the current classifcations. Some have not Aquatic Chronic 1, and one proposal has Carc. 2 (but not Muta. 1B and Repr. 1B) and STOT RE 2.	No

All other impurities are considered confidential (and are not relevant to the classification) and are only presented in the IUCLID-dossier section 1

Table 4: 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
Contains no additives					

Table 5: Test substances (non-confidential information) (this table is optional)

Toxicology-studies				
Study No.	**Study type**	**Batch No.**	**Production date**	**Purity**
RD-9119	A subchronic (3-month) oral toxicity study in the dog via capsule administration with Thiophanatemethyl	TIF-1016	20.06.1989	96.55 %
RD-9207	A chronic (1-year) oral toxicity study in the dog via capsule administration with Thiophanate-methyl	TIF-1016	20.06.1989	96.55 %
RD-00828N	Acute Neurotoxicity	TIF-1016	20.06.1989	99.7 %
RD-00634	Subchronic Neurotoxicity	TIF-1016	20.06.1989	99.7 %
RD-73052	Final report on the long-term oral toxicity studies of Thiophanate-methyl, Dimethyl 4,4'-o-phenylenbis (3-Thioallophanate) in beagle dogs for 24 months	ni	ni	ni
RD-73057	Final report on the chronic oral toxicity studies of Thiophanate-methyl, Dimethyl 4,4'-o-phenylenebis (3-Thioallophanate) in rats of spraguedawley strain for 24 months	ni	ni	ni
RD-02805	Phototoxicity	TDE-021P	21.05.2004	98.52 %
RD-73062	Mutagenic, cytogenetic and teratogenic studies on Dimethyl 4,4'OPhenylenebis (3-Thioallophanate), Thiophanate-methyl fungicide	ni	ni	ni
RD-9728	Thiophanate-methyl technical: induction of micronuclei in cultured human peripheral blood lymphocytes	TEE 2007	05.1995	98.23 %
RD-9729	Thiophanate-methyl technical: study to determine the threshold of action for the induction of aneuploidy in cultured human peripheral blood lymphocytes	TIF-1016	20.06.1989	96.39 %
RD-84109	Gene mutation in chinese hamster V 79 cells with Thiophanate-methyl	TM 767	26.02.1984	96.36%
RD-9329	Topsin-M - two generation oral (dietary administration) reproduction toxicity study in the rat (with one litter in the P and two liters in the F1 generation)	TIF-01016	ni	95.93 %
RD-9525	Final addendum histopathology report and peer review pathology report to MRID 42899101. Topsin-M – two generation oral (dietary administration) reproduction toxicity	TIF-01016	ni	95.93 %

Toxicology-studies				
Study No.	**Study type**	**Batch No.**	**Production date**	**Purity**
	study in the rat (with one litter in the p and two litters in the F1 generation)			
RD-9120	Mutagenicity test on Topsin M technical in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells	591	ni	95%
RD-8195	Evaluation of pure Thiophanate-methyl in the primary rat hepatocyte unscheduled DNA synthesis assay	CP 10B	ni	ni
RD-9160	21-Day dermal study in rabbits with Thiophanate-methyl technical	TIF-1016	ni	ni
RD-73058	The report on the carcinogenesis studies of Thiophanate-methyl, Dimethyl 4,4'-O-Phenylenebis (3-Thioallophanate) in mice of ICR-SLC strain for 24 months	ni	ni	ni
RD-9083	Thiophanate-methyl - acute oral toxicity study in rats	TIF-1016	Ni Takaoka Plant	96.55%
RD-9065	Thiophanate-methyl - reverse mutation study on bacteria	TIF-1016	Ni Takaoka Plant	96.55%
RD-9084	Thiophanate-methyl - acute dermal toxicity study in rabbits	TIF-1016	Ni Takaoka Plant	96.55%
RD-8711	Thiophanate-methyl - acute inhalation toxicity study in rats	TP-544	ni	95.3%
RD-8692	Thiophanate-methyl - primary dermal irritation study in rabbits	TM-948	ni	96.2%
RD-8691	Thiophanate-methyl - primary eye irritation study in rabbits	TM-948	ni	96.2%
RD-8924	Thiophanate-methyl - delayed contact hypersensitivity study in Guinea pigs	TM-948	ni	96.2%
RD-9059	Thiophanate-methyl - subchronic oral toxicity in rats	TIF-1016	Ni Takaoka Plant	96.55%
RD-9347	Topsin M - skin sensitization study in Guinea-pigs	TIF-1016	Ni Takaoka Plant	96.55%
RD-73055	Toxicological evaluation of Thiophanate-methyl (IV) - studies on the teratogenic effect of Thiophanatemethyl upon the fetus of ICR strain of mice	ni	ni	ni
RD-73059	Toxicological evaluation of Thiophanate-methyl (V) – some pharmacological properties of a new fungicide, Thiophanate-methyl	ni	ni	ni
RD-73051	Toxicological evaluation of Thiophanate-methyl (I) - acute and subacute toxicity of Thiophanatemethyl	ni	ni	ni
RD-73054	Toxicological evaluation of Thiophanate-methyl (III) - studies on the subchronic oral toxicity of Thiophanate-methyl in rats	ni	ni	ni
RD-73053	Toxicological evaluation of Thiophanate-methyl (II) - studies on the subchronic oral toxicity of Thiophanate-methyl in mice	ni	ni	ni
RD-73063	Effect of Thiophanate-methyl on reproductive function of multiple generations in the rat	NF-44	ni	ni
RD-9957	Thiophanate-methyl – mouse	TFB-2012	13.02.1996 Takaoka	97.28 %

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Toxicology-studies				
Study No.	**Study type**	**Batch No.**	**Production date**	**Purity**
	micronucleus test		Plant	
RD-8126	Teratology study of Thiophanatemethyl in rats	TM-123	ni	97.2 %
RD-8125	Pilot teratology study of Thiophanatemethyl in rats	TM-123	ni	97.2 %
RD-9327	Thiophanate-methyl – combined chronic toxicity/ oncogenicity study in rats	TIF-1016	ni Takaoka Plant	96.55 %
RD-9247	Thiophanate-methyl - metabolism in rats. Supplemental report to Nisso EC-338 (MRID#42474802)	TIF-1016	ni Takaoka Plant	96.55 %
RD-8642	Thiophanate-methyl - teratology study in the rabbit	TIF-1016	ni Takaoka Plant	96.55 %
RD-8642	Thiophanate-methyl - teratology study in the rabbit	TM-948	ni	96.2 %
RD-8641	Thiophanate-methyl - effects of oral administration upon pregnancy in the rabbit - 1. dosage range-finding study	TM-948	ni	96.2 %
RD-9328	18-month dietary oncogenicity study in mice with Topsin M	TIF-1016	ni Takaoka Plant	96.55 %
RD-9770	Oral (stomach tube) developmental toxicity study of Thiophanate-methyl in rabbits	TFB-2012	13.02.1996 Takaoka Plant	97.28 %
RD-9769	Oral (stomach tube and dietary) dosage-range developmental toxicity study of Thiophanate-methyl in rabbits	TFB-2012	13.02.1996 Takaoka Plant	97.28 %

Ecotoxicology studies				
Study No.	**Study type**	**Batch No.**	**Production date**	**Purity**
RD-00041	Toxicity to arthropods	ni	ni	ni
RD-II02074	The impact of Thiophanate-methyl on non-target flora and fauna	ni	ni	ni
RD-00554	Addendum report: toxicity of Thiophanate-methyl to green algae: calculation of EbC50- and ErC50-values	TM-502	ni	96.8%
RD-73066	Acute oral LD50 - bobwhite quail - topsin M technical	77-126-3	ni	94 %
RD-73067	Acute oral LD50 - mallard duck - topsin M technical	77-126-3	ni	94 %
RD-9330	Thiophanate-methyl - acute toxicity to rainbow trout (oncorhynchus mykiss) under flow-through conditions	TIF-01016	ni	95.93 %
RD-9651	Effect of Thiophanate-methyl on the mortality of the earthworm eisenia foetida	TEE 2007	ni	98.2 %
RD-02475	Toxicity to activated sludge	TEJ-096F	13.10.2005	98.08 %
RD-73068	Eight-day dietary LC50 – bobwhite quail - technical topsin M	35-135-3	ni	94 %
RD-73069	Eight-day dietary LC50 - mallard ducks - technical topsin M	35-135-3	ni	94 %
RD-9009	The prolonged toxicity of Thiophanatemethyl to rainbow trout (Salmo gairdneri)	TP-569	ni	95.2%
RD-9010	An assessment of the effects of Thiophanate-methyl on the reproduction of Daphnia magna	TP-569	ni	95.2%
RD-08328	Acute fish toxicity	TM-502	ni	96.8 %

RD-8328	Acute toxicity study of Thiophanatemethyl in rainbow trout	TM-502	ni	96.8 %
RD-8693	Thiophanate-methyl: acute toxicity study in Daphnids	TM-502	ni	96.8 %
RD-8304	Toxicity study of Thiophanate-methyl in green algae	TM-502	ni	96.8 %
RD-00011	Reproductive toxicity to birds	TIF-01016	ni	95.93 %
RD-00009	Reproductive toxicity to birds	TIF-01016	ni	95.93 %
RD-99127	Thiophanate-methyl - toxicity and reproduction study in bobwhite quail	TIF-01016	ni	95.93 %
RD-9229	Thiophanate-methyl - acute toxicity to Daphnids (Daphnia magna) under flow-through conditions (FIFRA Guideline no.: 72-2)	TIF-01016	ni	95.93 %
RD-84103	The acute toxicity of Thiophanatemethyl to the earthwom eisenia foetida	TM-803	ni	94.9 %
RD-00627	CM-0237 Acute Toxicity (LD50) to the Earthworm	31-02122	ni	98.6 %
RD-II 02438	Thiophanate-methyl: Inhibition of growth to the alga Pseudokirchneriella subcapitata	TBC–035G	10.2000 and 3.2002	99.7 % and 98.2 %
RD-02368	Toxicity to soil microflora	TDE-021P	21.5.2004	98.52 %
RD-99127	Reproductive toxicity to birds	TIF-01016	ni	95.93 %
RD-03266	Chronic toxicity to bees	TBC-035G	2003.2002	97.9 %
RD-02818	Fish early stage toxicity	TBC-035G	20.03.2002	97.9 %
RD-8652	Thiophanate-methyl - honey bee acute contact LD50	TM-948	ni	96.2%
	Toxicity to arthropods	ni	ni	ni

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	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	006-069-00-3	thiophanate-methyl (ISO) 1,2-di-(3-methoxycarbonyl-2-thioureido)benzene	245-740-7	23564-05-8	Acute Tox. 4* Skin Sens. 1 Muta. 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H317 H341 H400 H410	GHS07 GHS08 GHS09 Wng	H332 H317 H341 H410			
Dossier submitters proposal	006-069-00-3	thiophanate-methyl (ISO); dimethyl(1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate)	245-740-7	23564-05-8	Acute Tox. 4 Skin Sens. 1 Modify Muta. 1B Add STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	Retain H332 H317 Modify H340 Add H373 H400 H410	Retain GHS07 GHS08 GHS09 Modify Dgr	Retain H332 H317 Modify H340 Add H373 H410		M factor 10 M factor 10	
Resulting Annex VI entry if agreed by RAC and COM	006-069-00-3										

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data is 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	Hazard class not applicable	No
Flammable solids	Data is conclusive but not sufficient for classification.	Yes
Self-reactive substances	Data lacking	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data (experience in handling) is conclusive but not sufficient for classification.	Yes
Self-heating substances	Data lacking	No
Substances which in contact with water emit flammable gases	Data (experience in handling) is conclusive but not sufficient for classification.	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data is conclusive but not sufficient for classification.	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Harmonised classification proposed	Yes
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Thiophanate-methyl has a harmonised classification in Acute Tox. 4*, Skin sens. 1, Muta. 2, Aquatic Acute 1 and Aquatic Chronic 1. This has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC where it was classified as Xn; R20, R43, Muta. Cat 3; R68, R50-53 by the ECB as presented in Commission Directive 2001/60/EC.

The mutagenicity classification was discussed among the Specialised Experts in Arona on 1-2 September 1999. Thiophanate-methyl was considered together with the related compounds carbendazim and benomyl. Carbendazim and benomyl were classified in Cat 2 under DSD while for thiophanate-methyl "*the majority of the Specialised Experts stressed that the tabled information on toxicokinetic behaviour of thiophanate-methyl was supportive of a Category 3 classification. It was noted that the available documentation contained insufficient substantiation of the arguments in support of Category 2 raised by some Experts. In particular the rate of metabolism and excretion in the German monograph were not detailed enough to be taken into further consideration. … On account of the suspicion that the available data set was incomplete, the majority of the Specialised Experts recommended a preliminary classification with Muta Cat 3; R40 based on the currently available evidence and to make an effort to obtain additional data related to toxicokinetic behaviour and mutagenicity. The substance could be revisited if these attempts were successful.*"

RAC general comment
Thiophanate-methyl is an active substance used in plant protection products. The main uses in EU Member States are for agriculture, horticulture and viticulture.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Thiophanate-methyl is an active substance in the meaning of Regulation EC 1107/2009 and justification is not required (Article 36 CLP Regulation).

5 IDENTIFIED USES

Thiophanate-methyl is an active substance used in plant protection products. The main uses in EU Member States are intended for agriculture, horticulture and viticulture. The representative formulation Topsin M 500 SC is used as a fungicide on wine grapes, tomato, aubergine, leek, bean and wheat for the renewal of approval.

6 DATA SOURCES

Thiophanate-methyl was included in Annex I of Directive 91/414/EC by Commission Directive 2005/53/EC of 16 September 2005. Entry into Force of Annex I listing was 1st of March 2006. Annex I listing was extended to 31 October 2017 according to an amendment to Commission regulation No 540/2011 of 14 September 2012 (SANCO/10795/2013). Thiophanate-methyl is currently being evaluated under the following regulations for renewal of approval as an active substance in plant protection products:

• REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market

• COMMISSION IMPLEMENTING REGULATION (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

• COMMISSION IMPLEMENTING REGULATION (EU) No 533/2013 of 10 June 2013 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of the active substances 1-methyl-cyclopropene, chlorothalonil, chlorotoluron, cypermethrin, daminozide, forchlorfenuron, indoxacarb, thiophanate-methyl and tribenuron-methyl

The data presented in this dossier has been submitted by the applicant as part of the renewal process. Some of the data was submitted and evaluated during the first approval while other data was submitted for the first time for the purpose of renewal of approval. All data is presented in the Renewal Assessment Report (RAR) prepared by Rapporteur Member State (RMS) Sweden which has been submitted to EFSA.

The summaries of toxicity data provided in this dossier have largely been taken from Volume 1 of the RAR. Annex I to this report consists of the more extensive study summaries presented in Volume 3 of the RAR.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid (97%)	Nakayama, K., (1990), Report No. RD-09024 (111-001)	
Melting/freezing point	Decomposition before melting (165°C) (99.9%)	Nakayama, K., (1992), Report No. RD-09216 (112-001)	
Boiling point	Not relvant (see melting point)		
Relative density	No data		
Vapour pressure	> 9 x 10^{-6} Pa at 20°C (99.9%)	Higashida, S., Kobayashi, H. (1999), Report No. RD-IIM001 (115-005)	Direct measurement at specified temperature
Surface tension	72.2 mN/m at 20°C (86% of saturation concentration in water; 97.3%)	Tanaka, T. (2001), Report No. RD-II01069 (116-001)	
Water solubility	22.4 mg/L at 20°C (pH 4, phthalate buffer) (98.23%) 21.1 mg/L at 20°C (pH 5, phthalate buffer) (98.23%) 20.7 mg/L at 20°C (pH 6, phosphate buffer) (98.23%) 18.5 mg/L at 20°C (pH 7, phosphate buffer) (98.23%) 16.8 mg/L at 20°C (pH 7.5, phosphate buffer) (98.23%) unstable at pH > 8 at 20°C (98.23%) 24.6 mg/L at 25°C (pH 6.3 distilled, pH 6.3 saturated) (>99%) 21.8 mg/L at 25°C (pH 5.1 distilled, pH 5.2 saturated) (99%)	Gomyo, T. (1996), Report No. RD-09629 (114-002) Nakayama, K. (1992), Report No. RD-09219 (114-003) Nomura, O., Nakashima, N. (1987), Report No. RD-08775 (114-004) Soeda, Y., Shiotani, H. (1986), Report No. RD-08659 (114-007)	
Partition coefficient n-octanol/water	log P_{OW}= 1.40 at 25°C distilled water, pH was not	Shiotani, H. (1992), Report No. RD-	

Property	Value	Reference	Comment (e.g. measured or estimated)
	reported) (99.88%) log Pow = 1.53 (citrate-phosphate buffer, pH was not reported) log P_{OW} = 1.41 at pH 4 at 25°C (phthalate buffer) (98.23%) log P_{OW} = 1.45 at pH 5 (phthalate buffer) (98.23%) log P_{OW} = 1.47 at pH 6 (phthalate buffer) (98.23%)	09212 (114-005) Soeda, Y., Shiotani, H. (1986), Report No. RD-08660 (114-006) Gomyo, T. (1996), Report No. RD-09630 (114-008)	
Flash point	Not applicable. The active substance is a solid; its melting point is > 40 °C.		
Flammability	Not highly flammable (98.2%) In the preliminary test (EEC A.10) the flames extinguished immediately.	Krips, H.J. (1996), Report No. RD-09657 (142-001)	
Explosive properties	Not explosive in the sence of the test method (EEC A.14). (97.9% technical)	Pointer C. (2014), Report No. RD-02837 (141-002)	
Self-ignition temperature	No self-ignition (EEC A.16) of the test substance was observed (98.2%). The test substance had melted and changed into a black residue.	Krips, H.J. (1996), Report No. RD-09658 (142-002)	
Oxidising properties	The technical material is not oxidizing in the sence of the test method (97.9%). No test substance/cellulose burned to completion (EEC A.17).	Pointer C. (2014), Report No. RD-02837 (141-002)	
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	pKa = 7.28 at 25°C (>99%)	Ishihara, K. (1990), Report No. RD-09016 (115-003)	
Viscosity	Not relevant since the substance is a solid with a melting point >> 40 °C.		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14	Not explosive		Pointer C. (2014), Report No. RD-02837 (141-002)

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

One negative study performed in accordance with EEC A.14 was provided. It could also be added that the substance does not contain any functional groups known to confer explosive properties.

8.1.2 Comparison with the CLP criteria

It is not evident from the CLP-guidance that a negative result from the EEC A.14 automatically means that it should not be classified as an explosive under CLP. Neverthelss, based on the structure, it seems that the waiving criteria for non-testimng applies and a classification is thus not warranted.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed. Data is conclusive but not sufficient for classification.

RAC evaluation of Explosives

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on one negative EU method A.14 study and lack of any functional groups known to confer explosive properties.

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as explosives is warranted. Data is conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (thiophanate-methyl is not a gas)

8.3 Oxidising gases

Hazard class not applicable (thiophanate-methyl is not a gas)

8.4 Gases under pressure

Hazard class not applicable (thiophanate-methyl is not a gas)

8.5 Flammable liquids

Hazard class not applicable (thiophanate-methyl is not a liquid)

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Not highly flammable. In the preliminary test the flames extinguished immediately		Krips, H.J. (1996), Report No. RD-09657 (142-001)

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

One test performed in accordance with EEC A.10 was provided. The substabce did not ignite in the preliminary screening test and is thus not regarded a highly flammable in the sense of the test method.

8.6.2 Comparison with the CLP criteria

The preliminary screening test in EEC A.10 and in CLP are identical. The substance should thus not be classified as a flammable substance under CLP.

8.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

RAC evaluation of Flammable solids

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on one negative EU method A.10.

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as flammable solid is warranted. Data is conclusive but not sufficient for classification.

8.7 Self-reactive substances

Data lacking

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

No data has been provided addressing this property.

8.7.2 Comparison with the CLP criteria

No data has been provided that addresses this property. However, the structure of thiphanate-methyl does not contain any funtctional groups known to confer self-reactive properties (compared with Tables A6.1 and A6.2 in Appendix 6 to UN-MTC) . The waiving crtieria in CLP therefore applies and no classification for self-reactive properties is warranted.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed due to lack of data.

RAC evaluation of Self-reacting substances

Summary of the Dossier Submitter's proposal

No study results are available for this hazard class. However, according to the DS, the structure of thiophanate-methyl does not contain any functional groups known to confer explosive or self-reactive properties (compared with Tables A6.1 and A6.2 in Appendix 6 to UN- RTDG). The waiving criteria in CLP therefore applies and no classification for self-reactive properties is warranted.

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as self-reacting substance is warranted. Data is conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Hazard class not applicable (thiophanate-methyl is not a liquid).

8.9 Pyrophoric solids

Data lacking

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

No specific data derived in accordance with the recommended test method in CLP has been provided. However, thiophanate-methyl has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections)

8.9.2 Comparison with the CLP criteria

Based on experience in handling of thiophanate-methyl, it is not a pyrophoric solid (compare with example in CLP guidance section 2.10.7.2).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

RAC evaluation of Pyrophoric solids

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on the fact that thiophanate-methyl has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as pyrophoric solid is warranted with reference to CLP 2.10.4.1. Data is conclusive but not sufficient for classification.

8.10 Self-heating substances

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

Method	Results	Remarks	Reference
EEC A.16	No self-ignition of the test substance was observed. The test substance had melted and changed into a black residue.		Krips, H.J. (1996), Report No. RD-09658 (142-002)

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

One negative study conducted in accordance with EEC A.16 was provided.

8.10.2 Comparison with the CLP criteria

Since no study has been provided in accordance with the recommended test method in CLP a full assessment cannot be made.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed due to lack of data.

8.11 Substances which in contact with water emit flammable gases

Data lacking

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

No specific data derived in accordance with the recommended test method in CLP has been provided. However, thiophanate-methyl has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

8.11.2 Comparison with the CLP criteria

Based on experience in handling of thiophanate-methyl, it is not a substance which in contact with water emit flammable gases (compare with CLP guidance section 2.12.3.2).

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

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

RAC evaluation of Substances which in contact with water emit flammable gases

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on that thiophanate-methyl has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as a substances which in contact with water emit flammable gases pyrophoric solid is warranted with reference to CLP 2.12.4.1. Data is conclusive but not sufficient for classification.

8.12 Oxidising liquids

Hazard class not applicable (thiophanate-methylis not a liquid).

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	The technical material is not oxidizing in the sence of the test method. No test substance/cellulose burned to completion		Pointer C. (2014), Report No. RD-02837 (141-002)

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

One test performed in accordance with EEC A.17 was provided. None of the test material:cellulose mixtures (2:1, 1:1, 1:2) burned to completion. The reference mixture (barium nitrate:cellulose, 3:2) burned vigorously to completion in 29 seconds.

8.13.2 Comparison with the CLP criteria

The test under EEC A.17 do not utilize the same reference standard as in the test recommended under CLP (potassium bromate) and the ratio of test substance:cellulose mixture to be tested according to the decision logic in CLP is 1:1 and 4:1 whereas the available test only tested the 1:1 mixture (and 2:1, and 1:2). Nevertheless, since thiophanate-methyl only contains oxygen which is bonded to carbon or hydrogen the waiving criteria under CLP applies and the substance should not be classified for oxidising properties.

8.13.3 Conclusion on classification and labelling for oxidising solids

RAC evaluation of Oxidising solids

Summary of the Dossier Submitter's proposal

The DS presented one negative EU method A.17 study. The DS was aware that this test does not utilize the same reference standard as in the test recommended under CLP. However, thiophanate-methyl only contains oxygen which is bonded to carbon or hydrogen and thus 2.14.4.1 applies and the substance should not be classified for oxidising properties. The DS thus proposed no classification for oxidising solids.

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as oxidising solid is warranted with reference to CLP 2.14.4.1. The data is conclusive but **not sufficient for classification**.

8.14 No classification is proposed. Data is conclusive but not sufficient for classification.Organic peroxides

Hazard class not applicable (thiophanate-methyl is not a organic peroxide).

8.15 Corrosive to metals

No data has been provided addressing this property.

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

No data has been provided addressing this property. Thiophanate-methyl does contain acidic or basic functional groups (pka=7.3) and should thus be considered for classification in this class according to the CLP-guidance. However, the substance is a high metling substance which also means that it is currently difficult to test with the available test method.

8.15.2 Comparison with the CLP criteria

No data has been provided and a thorough assessment can thus not be made.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to lack of data.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

Test substance Test type Method Guideline	Route of exposure	Species Strain Sex No./group Vehicle	Exposure Period	Doses	Main findings	Reference
^{14}C-thiophanate-methyl Thiophanate-methyl EPA 85-1 GLP Acceptable	Oral (gavage)	Rat Fisher 344 M, F Group B, C, D, D2: 5/sex/group Group B2: 3 M.	Group B, B2[1], D and D2[2]: one single dose ^{14}C-thiophanate-methyl. Group C: one single dose thiophanate-methyl repeated for 14 days, then one single dose ^{14}C-thiophanate-methyl.	<u>Low dose:</u> Group B: 13 mg/kg bw. Group B2: 13.3 mg/kg bw. Group C: 14 mg/kg bw (thiophanate-methyl), 10 mg/kg bw (^{14}C-thiophanate-methyl) <u>High dose:</u> Group D: 140 mg/kg bw. Group D2: 171 mg/kg bw.	<u>Low dose (groups B, B2):</u> C_{max}: 1.7-4.2 µg eq/g T_{max}: 1-3 hours post dosing. $T_{1/2(initial)}$: 1.6-2.8 h Excretion: ~99.5% within 96 hours. Radioactivity mainly excreted with urine (70%) and faeces (30%) Main residues: 5-OH-carbendazim-S (urine), 4-OH-thiophanate-methyl (feces). Distribution: Highest residue levels in liver, thyroid and kidney. <u>Pre-administration low dose (group C):</u>	RAR Vol 3 B.6.1.1.1

[1] Group B2 was established since the metabolic pattern of group B slightly differed from that of group C.

[2] Group D2 was established since Group D due to technical difficulties was given only 140 mg/kg bw instead of the intended 170 mg/kg bw.

					C_{max}: 1.7-4.2 µg eq/g T_{max}: 1-3 hours post dosing. $T_{1/2(initial)}$: 1.6-2.8 h Excretion: ~99.5% within 96 hours. Radioactivity mainly excreted with urine (70%) and faeces (30%) Main residues: 5-OH-carbendazim-S (urine), thiophanate-methyl (faeces). Distribution: Highest residue levels in liver, thyroid and kidney. <u>High dose (groups D, D2):</u> C_{max}: 14-27 µg eq/g T_{max}: 2-7 hours post dosing $T_{1/2(initial)}$: 2.4-7.8 h Excretion: ~99.5% within 96 hours. Radioactivity mainly excreted with faeces (70%) and urine (30%). Main residues: 5-OH-carbendazim-S (urine), thiophanate-methyl (faeces). Distribution (96 h post dosing): Highest residue levels in liver, thyroid and kidney. No sex differences.	
^{14}C-thiophanate-methyl OECD 417 GLP Acceptable	Oral (gavage)	Rat Fisher 344/NCrHsd M, F 9/sex	One single dose	14 mg/kg bw	Blood, males(females): C_{max}: 4.7(4.8) µg eq/g T_{max}: 2 h $T_{1/2(initial)}$: 3.4(4.6) h $T_{1/2(terminal)}$: 12.4(10.5) h AUC_{inf}: 43(47) µg h/g Plasma: C_{max}: 5.9(5.4) µg eq/g T_{max}: 2 h $T_{1/2(initial)}$: 3.5(5.1) h $T_{1/2(terminal)}$: 8.7(8.9) h AUC_{inf}: 52(56) µg h/g Blood/plasma ratio: ~0.7 up to 24 h, thereafter ~1 at 48 h No sex differences.	RAR Vol 3 B.6.1.1.1
^{14}C-thiophanate-methyl OECD 417 GLP Acceptable	Oral (gavage)	Rat Fisher 344/NCrHsd M, F 6/sex	One single dose	14 mg/kg bw	Oral bioavailability: 89% Excretion: 99.6% complete after 48 h. Excretion was driven by urine (47%) and bile (40%). 7% was recovered from faeces. No sex differences.	RAR Vol 3 B.6.1.1.1
^{14}C-thiophanate-methyl Supportive	Oral	Rat Wistar M	One single dose	24.2 mg/rat (~65 mg/kg bw)	60% of the administered radioactivity was excreted via faeces. 30% was found in urine.	RAR Vol 3 B.6.1.1.1
^{14}C-thiophanate-	Oral (via diet)	Rat Wistar	Repeated dose 20 consecutive	42 ppm (~2.25 mg/kg bw)	Excretion: Each day ~90% of the ingested radioactivity was	RAR Vol 3 B.6.1.2.1

methyl Supportive		F 15	days		excreted in urine (54%) and faeces (35%). Distribution: Highest residue levels in GI-tract, thyroid, adrenals and liver.	
^{14}C-thiophanate-methyl Thiophanate-methyl EPA 85-1 GLP Acceptable	Oral (gavage)	Mice CD 1 M 5	One single high dose	171 mg/kg bw	Excretion: 99.9 within 48 hours. Radioactivity was excreted mainly with faeces (73%) and urine (26%). Main residues: 5-OH-carbendazim-S (urine), thiophanate-methyl (faeces). No significant differences in the relative excretion to faeces and urine when compared to rats.	RAR Vol 3 B.6.1.1.2
I: [^{14}C-thio-ureido]-thiophanate-methyl II: [^{35}S-thio-ureido]-thiophanate-methyl III: [^{14}C-methyl]-thiophanate-methyl IV: [^{14}C-phenyl]-thiophanate-methyl Supportive	Oral (gavage)	Mice dd-Y M	One single dose	I: 2 mg/mouse (~100 mg/kg bw) II: 1 mg/mouse (~50 mg/kg bw) III:3 mg/mouse (~150 mg/kg bw) IV: 1.1 mg/mouse (~55 mg/kg bw)	T_{max}: 2-3 h Excretion: Majority of radioactivity within 24 hours. Radioactivity was excreted mainly with urine (70-89%, depending on labelling) and faeces (18-29%, depending on labelling). Distribution (3 h post dosing): Highest residue levels in GI-tract, liver and kidney.	RAR Vol 3 B.6.1.1.2
^{14}C-thiophanate-methyl Supportive	Oral (capsule)	Dog Beagle	One single dose	9.85 mg/dog (~1 mg/kg bw)	Radioactivity was excreted mainly with urine (74%) and faeces (14%).	RAR Vol 3 B.6.1.1.3

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

The toxicokinetics of thiophanate-methyl was studied following oral exposure via gavage or feed in rats, mice and dogs following low and/or high dose administration. The data from the acute- and repeated dose toxicity studies indicated that toxicity following exposure to thiophanate-methyl via the dermal route was not of concern compared to oral administration, hence studies on absorption, distribution, metabolism and excretion following exposure via the skin is not required.

Time-concentration profile

The time-concentration profiles for thiophanate-methyl was investigated following oral administration of single low and high doses in male and female rats. Peak concentrations in blood were reached after 1-4 hours after administration. The majority (>99.5%) of the thiophanate-methyl related radioactivity was eliminated from blood within 48 (low dose) or 96 hours (high dose). The time-concentration profile of thiophanate-

methyl related radioactivity in blood and plasma after low dose administration is depicted in Figure 9.1.-1. Similar parameters were obtained in both male and female rats indicating that there are no sex differences in the toxicokinetics of thiophanate-methyl.

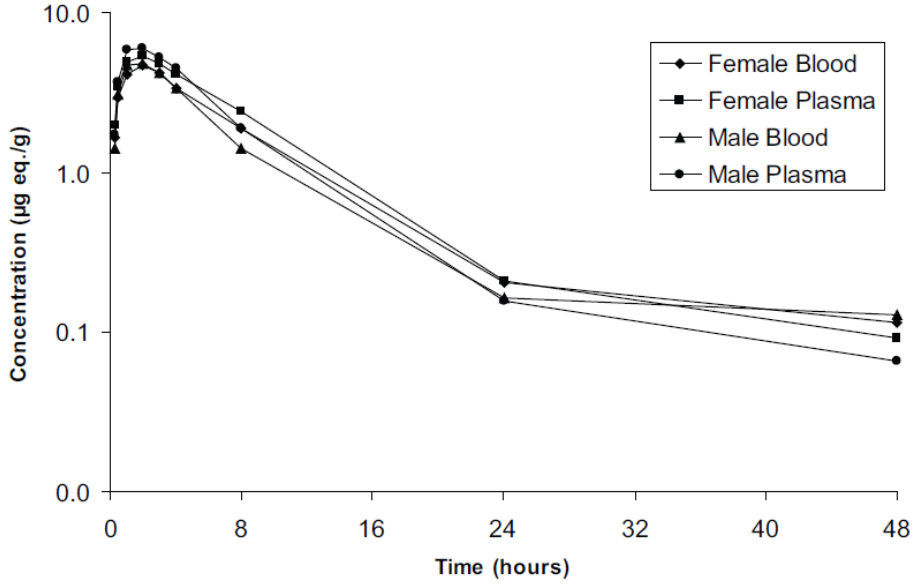


Figure 9.1.-1 Concentration-time profiles in blood and plasma after single oral low dose administration of ^{14}C-thiophanate-methyl.

Absorption

In rats, the absorption from the gastro-intestinal tract of low doses of thiophanate-methyl was rapid and represented approximately 89% of the administered dose, based on excretion in urine, bile and faeces. A clear shift from mainly urinary excretion at low doses to predominantly faecal excretion following high dose administration of thiophanate-methyl was detected, suggesting a decrease in oral absorption at high doses. The results from measurements of radioactivity in blood in the same studies provide some support to the statement - there seems to be a less than proportional increase of the AUC in relation to the increase in dose following high dose administration of thiophanate-methyl – but the level of detail in the studies was too low for any conclusion to be drawn. Another explanation to the observation may be that excretion of thiophanate-methyl via bile increases at high doses compared to low doses, meaning that the fraction thiophanate-methyl absorbed by the oral route would be independent of dose. Unfortunately, there are no studies available on excretion via bile following high dose administration of thiophanate-methyl to help elucidate this matter.

Distribution

Thiophanate-methyl is widely distributed in the body. In rats, at 96 hours post dosing (single low-, repeated low-, and single high dose administration) thiophanate-methyl related radioactivity was mainly found in thyroid, liver, and kidneys. Thiophanate-methyl was also present in testes and ovaries at levels above or similar to the ones in blood. In male mice, after administration of a single low dose, the highest radioactivity was found in liver, followed by kidney and blood. Low levels of radioactivity was also found in thyroid and testis.

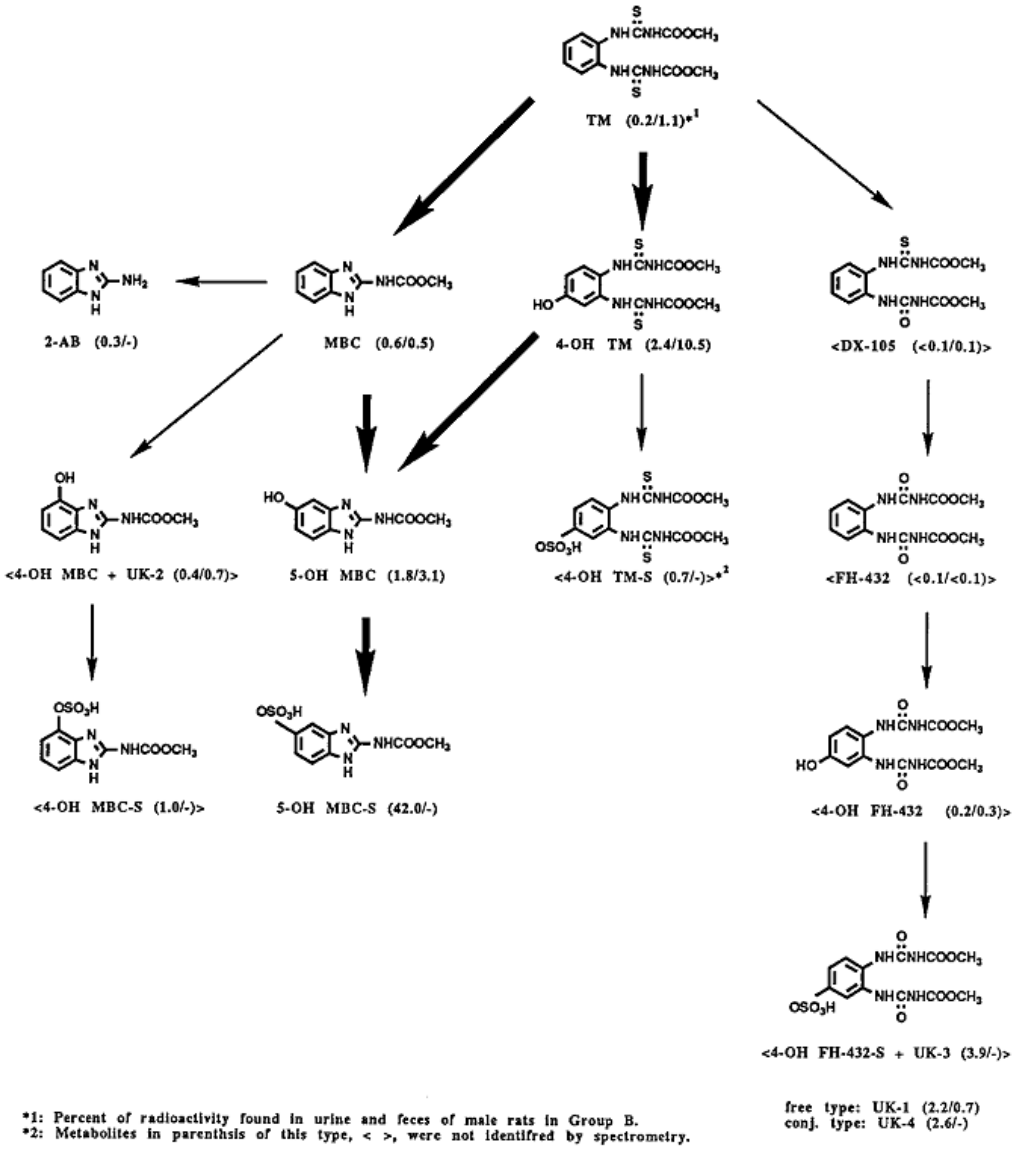
Metabolism

The results of the metabolism studies in rat showed that neither the dose level nor the duration of exposure resulted in a major change in the metabolites, identified in urine. The most important urinary metabolite at

both low and high doses was 5-OH-carbendazim-S which accounted for up to 42% of the total radioactivity following single low dose administration. The formation of 5-OH-carbendazim-S is proposed to occur via two competing pathways; one with carbendazim (MBC in table 9.1-2) as an intermediate. The proposed metabolic pathway of thiophanate-methyl in rats is depicted in Figure 9.1-2. The biotransformation routes in mice were found to be similar. In rats, there were an indication of possible gut microfloral enzyme induction, and/or possible biotransformation enzyme induction following repeated exposure, as indicated by the lower residue levels in excreta following repeated dosing relative to single low dose administration.

Excretion

Excretion of thiophanate-methyl in rats, mice and dogs occurred rapidly via urine and faeces. The excretion was almost complete within 24 to 96 hours depending on dose level and species, and the excretion patterns were comparable between the three species. Excretion via expired CO_2 was negligible in rats. At low doses, most thiophanate-methyl was excreted with urine (70% and 74 % in rats and dogs, respectively), and to a lesser extent via faeces (30% and 14% in rats and dogs, respectively9. After high dose administration the relationship was reversed and most thiophanate-methyl was recovered from faeces (70% and 73% in rats and mice, respectively), and to a lesser extent via urine (30% and 26% in rats and mice, respectively. During repeated low dosing of rats, every day during the treatment about 90% of the administered radioactivity was excreted via urine (54%) and faeces (35%). Hence, there is no indication that thiophanate-methyl accumulates in the body. Excretion via bile was investigated in one study in rats following single low dose administration. Excretion was found to be driven by both urine (47%) and bile (40%). Only 7% of the administered radioactivity was recovered from faeces.



MBC = carbendazim

Figure 9.1.-2. Proposed metabolic pathway of thiophanate-methyl in rats.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 14: Summary table of animal studies on acute oral toxicity

Test substance/ Route	Vehicle	Method Guideline	Species Strain Sex No./dose	Dose levels	LD_{50}/LC_{50}	Reference
Thiophanate-methyl Oral EPA OPP 81-2	Distilled water	EPA OPP 81-1 (similar to OECD 401)	Rat Crj:CD(SD) M, F 5/sex	5000 mg/kg bw	>5000 mg/kg bw	RAR Vol 3 B.6.2.1

M: male; F: female

Table 15: Summary table of human data on acute oral toxicity

No data.

Table 16: Summary table of other studies relevant for acute oral toxicity

No data.

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

An acute oral toxicity study was conducted according to GLP and EPA OPP 81-1. Five Crj:CD(SD) rats/sex received a single oral gavage treatment with thiophanate methyl in distilled water at 5000 mg/kg bw (10 mL/kg bw) and were then observed for 14 days. No mortalities or treatment-related changes were noted during the study period.

10.1.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), classification in Acute Tox. 4 (the lowest classification) is required for substances with oral LD_{50} of 300-2000 mg/kg bw. The LD_{50} for oral toxicity weas above 2000 mg/kg bw and thiophanate-methyl thus does not fulfil the classification criteria for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification is proposed for thiophanate-methyl.

10.2 Acute toxicity - dermal route

Table 17: Summary table of animal studies on acute dermal toxicity

Test substance/ Route	Vehicle	Method Guideline	Species Strain Sex No./dose	Dose levels	LD_{50}/LC_{50}	Reference
Thiophanate-methyl Dermal EPA OPP 81-2	Distilled water	EPA OPP 81-2 (similar to OECD 402)	Rabbit Kbs:JW 5/sex	2000 mg/kg bw	>2000 mg/kg bw	RAR Vol 3 B.6.2.2

M: male; F: female

Table 18: Summary table of human data on acute dermal toxicity

No data.

Table 19: Summary table of other studies relevant for acute dermal toxicity

No data.

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

An acute dermal toxicity study was conducted according to GLP and EPA OPP 81-2. Five 5 Kbs:JW rabbits/sex were treated topically with thiophanate methyl in distilled water at 2000 mg/kg bw, or with water alone (control group), for 24 hours under occlusive conditions (5 mL/kg bw), and were then observed for 14 days. No mortalities was noted during the study period. There was erythema on the treatment area for two days in seven rabbits. No other effects were noted.

10.2.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), classification in Acute Tox. 4 (the lowest classification) is required for substances with a dermal LD_{50} of 1000-2000 mg/kg bw. The LD_{50} for dermal toxicity weas above 2000 mg/kg bw and thiophanate-methyl thus does not fulfil the classification criteria for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed for thiophanate-methyl.

10.3 Acute toxicity - inhalation route

Table 20: Summary table of animal studies on acute inhalation toxicity

Test substance/ Route	Vehicle	Method Guideline	Species Strain Sex No./dose	Dose levels	LD_{50}/LC_{50}	Reference
Thiophanate-methyl Inhalation (4 hr, whole body, aerosol) EPA OPP 81-3	-	EPA OPP 81-3 (similar to OECD 403)	Rat Crj:CD(SD) M, F 5/sex	0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L	LC_{50} M: 1.7 mg/L LC_{50} F: 1.9 mg/L	RAR Vol 3 B.6.2.3

M: male; F: female

Table 21: Summary table of human data on acute inhalation toxicity

No data.

Table 22: Summary table of other studies relevant for acute inhalation toxicity

No data.

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

An acute inhalation toxicity study was conducted according to GLP and EPA OPP 81-3. Five Crj:CD(SD) rats/sex/group were exposed to a dust aerosol (MMAD: 3.7-4.5 µm) of thiophanate-methyl at concentrations of 0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L, or to clean air (control group), for 4 hours, and then observed for 14 days. Exposure was via whole body inhalation. During the study period, 5/5 males and 3/5 females died at 1.9 mg/L, and 1/5 females died at 1.0 mg/L. The calculated 4-hour LC_{50} values were 1.7 mg/L for males and 1.9 mg/L for females.

10.3.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP) , classification in Acute Tox. 4 (the lowest classification) is required for substances with an inhalation LC_{50} of 1.0-5.0 mg/L. The LC_{50} for inhalation toxicity was 1.7-1.9 mg/L and thiophanate-methyl thereby fulfils the criteria for classification with Acute Tox 4; H332.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification in **Acute Tox 4; H332** is proposed for thiophanate-methyl.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

In the CLH report, an acute oral toxicity study was reported. The study was conducted according to GLP and EPA OPP 81-1.

Five Crj:CD(SD) rats/sex received a single oral gavage treatment of thiophanate-methyl in distilled water at 5 000 mg/kg bw and were then observed for 14 days. No mortalities or treatment-related changes were noted during the study period, thus the ATE is > 5 000 mg/kg bw.

No classification for acute toxicity via the oral route was proposed for thiophanate-methyl by DS.

Comments received during public consultation

No specific comment on acute toxicity oral was received.

Assessment and comparison with the classification criteria

According to the CLP Regulation, ATE values above 2 000 mg/kg bw do not trigger classification. RAC agrees that **no classification is warranted for acute toxicity via the oral route**.

Summary of the Dossier Submitter's proposal

Acute toxicity: dermal

In the CLH report, an acute dermal toxicity study was reported. The study was conducted according to GLP and EPA OPP 81-2.

Five 5 Kbs:JW rabbits/sex were treated topically with thiophanate-methyl in distilled water at 2 000 mg/kg bw, or with water alone (control group), for 24 hours under occlusive conditions (5 mL/kg bw), and were then observed for 14 days. No mortalities was noted during the study period, therefore the ATE is > 2 000 mg/kg bw. There was erythema on the treatment area for two days in seven rabbits. No other effects were noted.

No classification for acute toxicity via the dermal route is proposed for thiophanate-methyl by DS.

Comments received during public consultation

No specific comment on acute toxicity dermal was received.

Assessment and comparison with the classification criteria

According to the CLP Regulation, ATE values above 2 000 mg/kg bw do not trigger classification. RAC agrees that **no classification is warranted for acute toxicity via the dermal route**.

Summary of the Dossier Submitter's proposal

Acute toxicity: inhalation

In the CLH report, an acute inhalation toxicity study was reported. The study was conducted according to GLP and EPA OPP 81-3.

Five Crj:CD(SD) rats/sex/group were exposed to a dust aerosol (MMAD: 3.7-4.5 µm) of thiophanate-methyl at concentrations of 0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L, or to clean air (control group), for 4 hours, and then observed for 14 days. Exposure was via whole body inhalation. During the study period, 5/5 males and 3/5 females died at 1.9 mg/L, and 1/5 females died at 1.0 mg/L. The calculated 4-hour ATE values were 1.7 mg/L for males and 1.9 mg/L for females. Several clinical signs were observed (e.g. tremors, convulsions, decreased motor activity), but at doses close to the

LC_{50} values.

Therefore, the DS proposed to confirm the current classification as Acute Tox. 4; H332 for thiophanate-methyl, without an ATE value.

Comments received during public consultation

Two commenting MSCA agreed with DS to retain the classification for Acute Tox. 4; H332. One of the MSCA proposed an ATE value of 1.7 mg/L, and the DS agreed.

Assessment and comparison with the classification criteria

According to the CLP Regulation, classification in Acute Tox. 4 is required for substances with an inhalation ATE of 1.0-5.0 mg/L. The ATE for inhalation toxicity of thiophanate-methyl is 1.7 and 1.9 mg/L for female and male respectively and thereby fulfils the criteria for classification as Acute Tox 4 H332. Therefore, RAC agrees with the DS that classification of thiophanate-methyl as **Acute Tox. 4; H332 with an ATE value of 1.7 mg/L (dusts and mists)** is warranted.

10.4 Skin corrosion/irritation

Table 23: Summary table of animal studies on skin corrosion/irritation

Test substance/ Route	Method Guideline	Species Strain Sex No./dose	Dose level	Remarks	Reference
Thiophanate-methyl Skin	EPA OPP 81-4 (similar to OECD 404)	Rabbit New Zealand M 6	500 mg thiophanate-methyl moistened with water	No skin reactions	RAR Vol 3 B.6.2.4

Table 24: Summary table of human data on skin corrosion/irritation

No data.

Table 25: Summary table of other studies relevant for skin corrosion/irritation

No data.

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

In a study performed in accordance with GLP and EPA OPP 81-4 (similar to OECD 404), 6 male New Zealand White rabbits each received dermal treatments with 0.5 g of thiophanate-methyl moistened with water for 4 hours under occlusive conditions, and skin reactions were then scored for erythema and oedema formation according to the Draize method at 0, 0.5, 1, 24, 48 and 72 hours. No skin reactions were observed.

10.4.2 Comparison with the CLP criteria

Not relevant as no effects were observed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed for thiophanate-methyl.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation based on one study performed in accordance with GLP and EPA OPP 81-4 (similar to OECD TG 404). Six male New Zealand White rabbits received dermal treatments with 0.5 g of thiophanate-methyl moistened with water for 4 hours under occlusive conditions, and skin reactions were then scored for erythema and oedema formation according to the Draize method at 0, 0.5, 1, 24, 48 and 72 hours. No skin reactions were observed at any time points.

Comments received during public consultation

One comment was received by a MSCA that agreed to no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

RAC agrees with the DS' assessment that no classification is warranted due to the lack of adverse effects in the study. The data is conclusive but **not sufficient for classification**.

10.5 Serious eye damage/eye irritation

Table 26: Summary table of animal studies on serious eye damage/eye irritation

Test substance/ Route	Method Guideline	Species Strain Sex No./dose	Dose level	Remarks	Reference
Thiophanate-methyl Eye	EPA OPP 81-5 (similar to OECD 405)	Rabbit New Zealand M 9	100 mg	1 h: Conjunctival redness in 7/9 rabbits (grade 1-2) 24 h: Conjunctival redness in 1/9 rabbits (grade 1)	RAR Vol 3 B.6.2.5

Table 27: Summary table of human data on serious eye damage/eye irritation

No data.

Table 28: Summary table of other studies relevant for serious eye damage/eye irritation

No data.

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

In a study performed in accordance with GLP and EPA OPP 81-4 (similar to OECD 405), 0.1 g of thiophanate-methyl was placed in the conjunctival sac of the left eye of 9 male New Zealand White rabbits. The treated eyes of 3 rabbits were washed 2 minutes after the treatment for 30 seconds, and then irritation was scored at 1, 24, 48 and 72 hours after treatment. Conjunctival redness (grade 1-2) was seen in 7/9 rabbits (grade 1-2) at the 1-hour reading and in 1/9 rabbits at the 24-hour reading (in the eye irritation study).

10.5.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP)table 3.3.2, a substance should be classified in Category 2 (irritating to eyes) if it produces *"at least in 2/3 animals, a positive response of:*

-corneal opacity ≥1 and/or

-iritis ≥1 and/or 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 installation of the test material, and which fully reverses within an observation period of 21 days."

These criteria were not fulfilled in the study performed with thiophanate-methyl.

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

No classification is proposed for thiophanate-methyl.

RAC evaluation of serious eye damage/eye irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for serious eye damage/eye irritation based on one study performed in accordance with GLP and EPA OPP 81-5 (similar to OECD TG 405). In the study, 0.1 g of thiophanate-methyl was placed in the conjunctival sac of the left eye of 9 male New Zealand White rabbits. The treated eyes of 3 rabbits were washed 2 minutes after the treatment for 30 seconds, and then irritation was scored at 1, 24, 48 and 72 hours after treatment in all animals. Conjunctival redness was seen in 7/9 rabbits (grade 1-2) at the 1-hour reading and in 1/9 rabbits at the 24-hour reading.

The DS argued that the effects seen were not sufficient for classification.

Comments received during public consultation

One commenting MSCA agreed with no classification for serious eye damage/eye irritation.

Assessment and comparison with the classification criteria

RAC agrees with the DS' assessment that no classification for serious eye damage/eye irritation is warranted due to that the adverse effects seen in the study do not fulfil the CLP criteria. The data is conclusive but **not sufficient for classification**.

10.6 Respiratory sensitisation

No data.

10.7 Skin sensitisation

Table 29: Summary table of animal studies on skin sensitisation

Test substance Test type Method Guideline	Route of exposure	Species Strain Sex No./group Vehicle	Exposure Period	Doses	Main findings	Reference
Thiophanate-methyl GPMT Acceptable	Intradermal /Dermal	Guinea pig Hartley F 10/group Freund's complete adjuvant/White vaseline.	The duration of dermal induction was 48 hours, carried out 7 days after intradermal induction. The duration of challenge was 24 hours, carried out another 14 days later. The experiment was repeated under the same conditions.	Intradermal induction: 3.5% (mildly irritant). Dermal induction: 42% (non-irritant). Challenge: 42% (non-irritant).	Challenge: 10/10 animals with positive skin reactions. Repeated study: Challenge: 10/10 animals with positive skin reactions. Sensitiser.	RAR Vol 3 B.6.2.6
Thiophanate-methyl Supportive	Intradermal	Guinea pig Hartley 14 Aqueous Arabic gum suspension.	Intradermal: 10 applications (3 per week). Challenge: 14 days after induction in a treated and non-treated area.	Intradermal induction: 1% Intradermal challenge: 1%	Diameter of erythema caused by sensitisation was slightly bigger than erythema caused by primary irritation. Weak sensitiser.	RAR Vol 3 B.6.2.6

Table 30: Summary table of human data on skin sensitisation

No data.

Table 31: Summary table of other studies relevant for skin sensitisation

No data.

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

The skin sensitisation potential of thiophanate-methyl was assessed in two studies in guinea pig. Both studies showed that thiophanate-methyl have skin sensitising properties. In the guinea pig maximization test (GPMT), considered acceptable, 100% of the animals showed positive skin reactions following intradermal induction to thiophanate-methyl. When the study was repeated, the response rate was identical.

The second test was considered supportive due to limitations in scope and reporting. The study was not performed in accordance with GLP nor with any specific guideline. It measured the diameter of erythema caused by sensitisation copmpared to erythema caused by primary irritation. The response rate was not reported. The diameter of erythema caused by thiophanate-methyl was slightly bigger than that of the erythema caused by primary skin irritation. A big difference (up to 4-fold) was noted for the positive control. Thiophanate-methyl showed weak sensitising properties under the conditions of the study. The weaker reactions in the second test may be due to the use of lower induction and challenge doses and/or the use of a different vehicle (Aqueous Arabic gum suspension compared to FCA). Thiophanate-methyl has currently a harmonised classification as a skin sensitiser (Skin Sens. 1, H317).

10.7.2 Comparison with the CLP criteria

The Regulation (EC) No 1272/2008 (CLP) allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. For Category 1, when an adjuvant Guinea pig test method is used, a response in at least 30% of the animals is considered positive. This criteria is fulfilled by thiophanate-methyl which had a response rate of 100% following the use of a 3.5% intradermal induction dose. Classification into sub-categories is only allowed if data are sufficient (CLP Annex I 3.4.2.2.1.1). The criteria for sub-categorisation based on results from Guinea pig maximisation tests are given below.

Table 32: Criteria for sub-category classification of skin sensitisers.

Sub-category	Assay	Response
1A	Guinea Pig Maximisation Test	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
1B	Guinea Pig Maximisation Test	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose

According to Table 43, thiophanate fulfils the criteria for subcategorisation in category 1B (≥ 30 % responding at > 1 % intradermal induction dose). However, the Guidance on the application of the CLP criteria, section 3.4.2.2.2, states that subcategorisation in 1B is only allowed if category 1A can be excluded. For thiophanate-methyl, there is a very high response rate following the use of a high intradermal induction dose. It is therefore possible that the use of a lower dose would result in a response rate which fulfills the criteria for category 1A. Subcategorisation of thiophanate-methyl is therefore not proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Thiophanate-methyl has a harmonised classification as a skin sensitiser (**Skin Sens. 1, H317**). No change is proposed.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Two guinea pig maximization tests (GPMT) are reported in the CLH dossier. Both studies showed that thiophanate-methyl has skin sensitising properties.

In the GPMT study, considered acceptable (GLP, guideline compliant, OECD TG 406) 100 % of the animals showed positive skin reactions following intradermal induction (induction conc. 3.5 %) to thiophanate-methyl. When the study was repeated, the response rate was identical.

The second GPMT study was considered supportive due to limitations in scope and reporting as the study was not performed in accordance with GLP nor following any specific guideline. The diameter of erythema caused by sensitisation was measured and compared to the erythema caused by primary irritation. The response rate was not reported. The diameter of erythema caused by thiophanate-methyl was slightly bigger than that of the erythema caused by primary skin irritation. A big difference (up to 4-fold) was noted for the positive control. Thiophanate-methyl showed weak sensitising properties under the conditions of the study. The weaker reactions in this test compared to the previous may be due to the use of lower induction and challenge doses (both 1 %) and/or the use of a different vehicle (Aqueous Arabic gum suspension compared to FCA).

Thiophanate-methyl has currently a harmonised classification as skin sensitizer (Skin Sens. 1; H317). The DS considered that the submitted studies confirmed the classification and therefore they did not propose changes.

Comments received during public consultation

One commenting MSCA agreed to retain classification as skin sensitiser 1.

Assessment and comparison with the classification criteria

In the acceptable submitted study on guinea pig on thiophanate-methyl a response rate of 100% following the use of a 3.5 % intradermal induction dose was observed. According to the Table 3.4.4 of the CLP Regulation, thiophanate fulfils the criteria for sub-categorisation in category 1B (≥ 30 % responding at > 1 % intradermal induction dose). However, the guidance on the application of the CLP criteria, section 3.4.2.2.2, states that sub-categorisation as 1B is only applicable if sub-category 1A can be excluded.

In this specific case, for thiophanate-methyl, there is a very high response rate following the use of a high intradermal induction dose. It is therefore possible that the use of a lower dose would result in a response rate which fulfils the criteria for sub-category 1A. Sub-categorisation of thiophanate-methyl is therefore not proposed.

Thiophanate-methyl has a harmonised classification as a **skin sensitiser (Skin Sens. 1; H317)**. RAC considers that no change of the existing classification is warranted.

10.8 Germ cell mutagenicity

Table 33: Summary table of mutagenicity/genotoxicity tests in vitro

Test substance Test system Method	Organism/Strain(s)	Concentrations tested (range)	Result		Remark	Reference
			+S9	-S9		
Thiophanate-methyl Bacterial Reverse Mutation Test (Ames test) OECD TG 471	*Salmonella typhimurium* TA100 TA1535 TA1537 TA98 *Escherichia coli* WP2uvrA	39.1 - 5000 µg/plate	Negative	Negative		RAR Vol 3 B.6.4.1.1 Doc. No. 557-006
Thiophanate-methyl Mammalian Cell Gene Mutation Test OECD TG 476	HPRT locus V79 Chinese hamster cells	6.25 - 100 µg/ml	Negative	Negative		RAR Vol 3 B.6.4.1.2 Doc. No. 557-003
Thiophanate-methyl (Topisn M*) Mammalian Chromosome Aberration Test OECD TG 473	Chinese hamster ovary cells	100 - 400 µg/ml (without S9) 250 – 1000 µg/ml (with S9-mix)	Negative	Negative		RAR Vol 3 B.6.4.1.3 Doc. No. 557-004
Thiophanate-methyl Unscheduled DNA Repair Synthesis (UDS test) OECD TG 482	Rat primary hepatocytes	5 – 1000 µg/ml	Negative	Negative		RAR Vol 3 B.6.4.1.4 Doc. No. 557-005
Thiophanate-methyl Micronucleus test Similar to OECD 487	Human peripheral lymphocytes	Up to 47.5 µg/mL (-S9 mix) Up to 550 µg/mL (+S9 mix)	Negative	Positive	Evidence for aneuploidy (-S9 mix)	RAR Vol 3 B.6.4.1.5 Doc. No. 557-001
Thiophanate-methyl Micronucleus test Examination of four chromosomes No guideline	Human peripheral lymphocytes	Range-finding study: 8.5 - 200 µg/mL Main study: 0.05 - 39.0 µg/mL (-S9 mix)	Not investi-gated	Positive	The purpose of the study was to establish a threshold for aneugenicity. Result: the threshold was found to be 6 µg/mL.	RAR Vol 3 B.6.4.1.6 Doc. No. 557-002

* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95%.

Table 34: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Test substance Test system Method	Species Strain Sex No./group	Frequency of application	Dose levels	Results	Remark	Reference
Thiophanate-methyl Chromosomal aberration (micronucleus test) OECD 474 Acceptable	B6D2F1 mice M, F 5/group	Single dose	500, 1000, 2000 mg/kg bw	Positive	The incidence in micronuclei was 4-fold higher than in the concurrent negative control group, but 6-fold lower than in the positive control group exposed to carbendazim.	RAR Vol 3 B.6.4.2.1 Doc. No. 557-007 Study evaluated in DAR
Thiophanate-methyl Chromosomal aberration (micronucleus test) OECD 474 Low reliability	Crl:CD-1 (ICR) mice M 5/group	Administered twice at an interval of approximately 24 hours	500, 1000, 2000 mg/kg bw	Negative	Data confirming bone marrow exposure not presented. There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.	RAR Vol 3 B.6.4.2.2 Doc. No. 557-016 Study submitted for the purpose of renewal
Thiophanate-methyl *In vivo* genotoxicity studies: micronucleus test, chromosome aberration analysis, comet assay Supportive	Lizards (*Podarcis sicula*) captured in the wild Control group 9 M & 9 F Exposed groups 10 M & 10 F	Administered by spraying twice weekly	100 ml of 1.5 % thiophanate-methyl solution used for spraying twice weekly during 15, 30 or 40 days.	Positive for all genetoxic effects tested	Information on the purity of the test substance is not available. The results may be relevant as supportive information for the assessment of the genotoxic potential of thiophanate-methyl.	RAR Vol 3 B.6.4.2.3 Doc. No. (892-017) Study submitted for the purpose of renewal
Thiophanate-methyl *In vivo* cytogenetic assay (i.p) Dominant lethal assay (i.p) Supportive	Wistar rats ICR mice Cytogenicity assay:30m/dose DL: 10m/dose group/week mated with 3 females. Repeated 8 weeks (240 females in total)	Cytogenicity assay: 5 d DL:single dose	Cytogenicity assay: 0, 62.5, 125, 250, 500 and 1000 mg/kg bw DL: 0, 8, 40, 200, 400 and 500 mg/kg bw	Cytogenicity assay: Inconclusive DL: Inconclusive	Deficiencies with respect to reporting (lack of individual data) and study design (lack of positive controls)	RAR Vol 3 B.6.4.3.1 Doc. No. RD-73062 (551-006) Study evaluated in DAR
Thiophanate-methyl Spermatogonial chromosomal abberation test Low reliability	Crl:CD-1 (ICR) mice M 6/group (5 analysed for chromosomal aberrations)	Single dose	500, 1000, 2000 mg/kg bw	Negative	Data confirming target cell exposure not presented. There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl.	RAR Vol 3 B.6.4.3.2 Doc. No. RD-03956 (557-012) Study submitted for the purpose of renewal

Thiophanate-methyl Spermatogonial chromosomal abberation test (Pretest) Low reliability	Crl:CD-1 (ICR) mice M 10/group for negative control and test substance, 4/group for positive control/group	Single dose	2000 mg/kg bw	Negative	Data confirming target cell exposure not presented. There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl. Non-GLP compliant test Guideline: not applicable	RAR Vol 3 B.6.4.3.2 RD-03888 (557-018) Study submitted for the purpose of renewal
Thiophanate-methyl Micronucleus test in germ cells No guideline Low reliability	Crl:CD-1 (ICR) mice M 5/group	Single dose	500, 1000, 2000 mg/kg bw	Negative	Data confirming target cell exposure not presented. There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl. In addition, micronuclei in erythrocytes were scored according to OECD 474; no increase in micronucleated polychromatic erythrocytes was observed	RAR Vol 3 B.6.4.3.3 No. RD-10093 (557-017) Study submitted for the purpose of renewal
Thiophanate-methyl Micronucleus test in germ cells (Pretest) No GLP No guideline Low reliability	Crl:CD-1 (ICR) mice M 4 or 5/group	Single dose	2000 mg/kg bw	Negative	Data confirming target cell exposure not presented. There is reason to be concerned about the sensitivity of the strain used to detect mutagenic effects of thiophanate-methyl. Two of the four positive control substances did not induce an increase in the frequency of micronucleated spermatids.	RAR Vol 3 B.6.4.3.3 No. RD-03931 (557-011) Study submitted for the purpose of renewal

Table 35: Summary table of human data relevant for germ cell mutagenicity

No data.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The gentotoxicity studies performed with thiophanate-methyl are thoroughly described in Annex I (Volume 3 to the RAR).

Thiophanate-methyl has been investigated for genotoxicity *in vitro* and *in vivo*.

Thiophanate-methyl tested negative in the bacterial reverse mutation test (Ame's test), mammalian cell gene mutation test, chromosome aberration test and unscheduled DNA synthethis test, however the *in vitro* micronucleus test was positive. A follow-up study was made to establish a threshold effect for the aneugenic effect noted in the micronucleus test.

The *in vivo* studies also indicate an aneugenic potential, as concluded at the first approval and which is in accordance with the current classification in Muta. 2 for aneugenicity in somatic cells. However, the DS is of the opinion that there is also evidence of clastogenicity.Information with some relevance for genotoxicity was found in the literature search performed by the applicant (see Vol 3, B.6.10). Data consists of a non-GLP study including a combined comet assay, micronucleus test and chromosome aberration test performed with thiophanate-methyl in lizards (Podarcis sicula). In the study lizards captured in the wild were sprayed twice weekly with a 1.5 % concentration of thiophanate-methyl (in water) for 15, 30 or 40 days and the results obtained indicated a potential for clastogenicity, since DNA damage and chromosome aberrations due to single or double strand breaks were increased compared to controls and correlated with exposure time. Due to the lack of a specification or information on the purity of the test substance it is not possible to exclude that effects could be due to any impurities present in the solution. Therefore, this study alone is considered to be of limited value for the hazard assessment.

However, stronger evidence for a clastogenic effect of thiophanate-methyl is available in the first mouse micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). The results of the study are presented in the study summary in Annex I and are summaried below:

Table 36: Incidence of micronucleated cells per 2000 immature erythrocytes examined

Sampling time	Treatment (compound)	Dose (mg/kg bw)	Incidence
24 hours	Vehicle control	0	1.3
	Thiophanate-methyl	500	4.2**
	Thiophanate-methyl	1000	3.8**
	Thiophanate-methyl	2000	6.3**
	Carbendazim	1000	24.5**
48 hours	Vehicle control	0	1.2
	Thiophanate-methyl	500	3.1**
	Thiophanate-methyl	1000	5.0**
	Thiophanate-methyl	2000	5.6**

** Statistically significant (nonparametric method of analysis, based on permutation with one-sided probabilities); $p < 0.001$

Table 37: Summary of centromeric staining results

Treatment (compound)	Dose (mg/kg bw)	Centromere-positive micronuclei (indicative of aneugenicity)	Centromere-negative micronuclei (indicative of clastogenicity
Thiophanate-methyl	2000	34 %	66 %
Carbendazim (positive control aneugenicity)	1000	68 %	32 %
Mitomycin C (positive control clastogenicity)	12	24 %	76 %

This was concluded by the DS after re-evaluation of the results, as presented below. Not presented in the previous version of this document, nor in the DAR, this study included centromeric staining of micronuclei, enabling a distrinction between a clastogenic effect (centromere-negative micronuclei) and an aneugenic effect (centromere-positive micronuclei). The proportion of centromere-negative micronuclei observed

following exposure to carbendazim, thiophanate-methyl and mitomycin C, respectively, was 32%, 66% and 76%, respectively. The result demonstrates that the proportion of centromere-negative micronuclei observed after thiophanate-methyl exposure was very close to the proportion observed after exposure to the known clastogen mitomycin C and distinctly different from the proportion observed after exposure to the known aneugen carbendazim. The result is a strong piece of evidence that thiophanate-methyl has clastogenic properties.

A new micronucleus study of thiophanate-methyl in the bone marrow of mice (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016) was submitted by the applicant as additional data during the Pesticide Peer Review (Evaluation table, data requirement 2.2). Since no statistically significant increase or dose-dependent relationship were observed in micronucleated polychromatic erythrocytes, the study author concluded that the result of the study was negative. However, no data demonstrating bone-marrow exposure were presented and, therefore, the negative result of the study is considered to be of low reliability. The reliability can be further questioned on account of the 4-fold increase in the incidence of micronucleated polychromatic erythrocytes observed in the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). This study used the mouse strain B6D2F1 and, in addition to the increase in micronuclei, substantial amounts of thiophanate-methyl and carbendazim were established in blood plasma following administration of thiophanate-methyl. In the new study a different mouse strain was used (Crl:CD-1 (ICR)), and the negative result obtained gives reason be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl.

In connection with this analysis it is relevant to mention that the applicant is of the opinion that the result of the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007) should not be concluded to be positive, since the increase in micronuclei might be within the range of historical negative control values. The view of the RMS is that the concurrent negative control group has the strongest weight in comparisons with results in exposed groups. In studies where a small increase was observed in one exposed group only, i.e. the result was not reproducible, it would be relevant to compare the result with historical negative control values to conclude if the observed increase should be considered to have occurred by chance only. However, in the first study (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016), a 4-fold increase in micronuclei was observed both in the experiment using 24 h sampling time and the experiment using 48 h sampling time. Moreover, in both experiments the increases in micronuclei were dose related. This clearly demonstrates that the observed effect occurred as a result of exposure to thiophanate-methyl and was not a chance finding. Accordingly, the new study (RAR Vol 3, B.6.4.2.2 Doc No. 557-016) should be ascribed low wheight in the evaluation of the genotoxicity of thiophanate-methyl.

As thiophanate-methyl has been demonstrated to be mutagenic in the bone marrow of mice, with evidence for both aneugenic and clastogenic effects, this effect can be suspected to occur also in germ cells. Therefore, an assessment of the potential of thiophanate-methyl to induce mutations in germ cells is considered necessary and is made below.

A dominant lethal test performed as part of a mutagenic, cytogenetic and teratogenic study is available. In the cytogenicity part of the study, metaphase chromosomes from the bone marrow as well as from spermatogonial cells were investigated, with no indication of an increase in chromosome aberrations. Neither was there an indication of treatment-related dominant lethal mutations in male mice. However, the study suffered from many deficiencies including the lack of individual data and a positive control to verify appropriate functioning of the test. Thus, based on this result, it is difficult to exclude that the aneugenic effects of thiophanate-methyl observed in somatic cells would not occur in germ cells. Overall, the result is considered inconclusive.

Although the dominant lethal assay is recommended as a method to follow up positive results observed in in vivo genotoxicity tests in somatic cells, the test is generally regarded to have low sensitivity and this is even recognised in the OECD guideline 478. Furthermore, it is noted that carbendazim, which is concluded to be a germ-cell mutagen (currently classified Muta. 1B, see section on classification) was also negative in dominant lethal tests (DRAR on carbendazim, 2009).

Additional data was requested by EFSA during the Peer review (Evaluation table, data requirement 2.2) and consisted of four new studies testing mutagenic effects of thiophanate-methyl in germ cells of mice, one spermatogonial chromosome aberration study with a preceding pretest and a micronucleus study in germ

cells with a preceding pretest. All four studies were concluded to be negative by the study author, since no statistically significant increase or dose-dependent relationship were observed in chromosome aberrations or micronuclei. However, no data demonstrating target-cell exposure were presented and, therefore, the negative results of the studies have low reliability. The reliability can be further questioned by the arguments presented above for the new bone marrow micronucleus test by (RAR Vol 3, B.6.4.2.2 Doc. No. 557-016), i.e. that the strain Crl:CD-1 (ICR) was used and that there is reason to be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl. Accordingly, all four studies should be ascribed low wheight in the evaluation of the genotoxicity of thiophanate-methyl in germ cells.

The applicant also performed a specific literature search only focused at aneugenicity (see RAR Vol 3, B.6.10 in Annex I). No information was found.

10.8.2 Comparison with the CLP criteria

At present, thiophanate-methyl is classified as Muta. 2; H341 based on a translation of the classification Muta. Cat. 3 established under Council Directive 67/548/EEC, now replaced by Regulation (EC) No 1272/2008. Carbendazim is classified as Muta. 1B; H340.

Criteria for Classification in category 1A can only be fulfilled if results are based on:

- *positive evidence from human epidemiological studies*.

This type of data is not available for thiophanate-methyl thus **classification in category 1A is not supported**.

Classification in **category 1B** is based on:

-positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; 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; 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.

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; or

-Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Considering that there is no robust data on germ cell genotoxicity available for thiophanate-methyl, it may be argued that the existing data do not fulfil the criteria for classification in category 1B. However, in the Guidance on the Application of the CLP Criteria Version 4.1 – June 2015 (and in the current draft of this guidance document in the ongoing updating process) the following is stated: "*It could be argued that in a case where in vivo mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, then that substance should also be considered as a Category 1B mutagen. Germ cell mutagens as the spermatogonia are generally not protected from substance exposure by the blood-testes barrier formed by the Sertoli cells. In such circumstances the relevant criteria are as follows:*

-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****"* (text highlighted in bold by the RMS is of particular relevance for the classification of thiophanate-methyl, as discussed below).

With respect to the above it is of importance to note that, according to data in the section on toxicokinetics (Vol 3, section B.6.1.1.1), low levels of labelled "parent equivalents" (i.e. below 0.5 ppm but in a range comparable to residue levels in the blood) are found in testis and ovaries of rats administered a single dose thiophanate-methyl. In rats administered repeated doses of the substance the levels in ovaries were initially higher (at 3 hours) than the levels in blood. Exposure of testis was also demonstrated in mice receiving a single dose of thiophanate-methyl. Overall, this indicates that gonads are exposed to the substance or its metabolites but no accumulation seems to occur. Moreover, according to data in the section on toxicokinetics, urinary metabolites carbendazim and 5-OH-carbendazim-S are formed to an extent of ≤1% and 42% of the total administered radioactivity respectively. It is reasonable to conclude that thiophanate-methyl, which is systemically available, detected in the gonads of rats and mice, and mutagenic (clastogenic and aneugenic) in the bone marrow of mice after oral administration has the ability to interact with the genetic material of germ cells.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Thiophanate-methyl is currently classified Muta. 2; H341 based on a translation from the classification established under the Dangerous Substances Directive. As the substance is mutagenic (clastogenic and aneugenic) *in vivo* in somatic cells (bone marrow), systemically available and detected in gonads of rats and mice, the data is considered to indicate "the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells" and classification of thiophanate-methyl in **Muta. 1B – H340** is therefore proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Thiophanate-methyl has been investigated for genotoxicity *in vitro* and *in vivo*.

It tested negative in the bacterial reverse mutation test (Ames test), mammalian cell gene mutation test, chromosome aberration test and unscheduled DNA synthesis test, but an *in vitro* micronucleus test in cultured human peripheral lymphocytes was positive with indications of an aneugenic effect. A follow-up study in the same experimental system reported that the threshold for the aneugenic effect *in vitro* was 6 µg/mL and the no effect level was 4 µg/mL.

In the CLH report the DS was originally of the opinion that there was also evidence of clastogenicity. Information with some relevance for genotoxicity was found in the literature search performed by the applicant.

A non-GLP study, including a combined comet assay, micronucleus test and chromosome aberration test, performed with thiophanate-methyl in lizards (*Podarcis sicula*) reported evidence of DNA damage and chromosome aberrations. However, due to the lack of information on the purity of the test substance, it was not possible to exclude that effects could be due to any impurities present in the solution and therefore this study was considered of limited value.

Stronger evidence for a clastogenic effect of thiophanate-methyl came from the first mouse micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). This study included centromeric staining of micronuclei, enabling a distinction between a clastogenic effect (centromere-negative micronuclei) and an aneugenic effect (centromere-positive micronuclei). The proportion of centromere-negative micronuclei observed following exposure to carbendazim, thiophanate-methyl and mitomycin C was 32 %, 66 % and 76 %, respectively, indicating a proportion of centromere-negative micronuclei observed after thiophanate-methyl exposure close to the proportion observed after exposure to the known clastogen mitomycin C and distinctly different from the proportion observed after exposure to the known aneugen carbendazim. The DS considered this result as an evidence of the clastogenic potential of thiophanate-methyl.

A new micronucleus study of thiophanate-methyl in the bone marrow of mice (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016) was submitted by the applicant as additional data during the Pesticide Peer Review (Evaluation table, data requirement 2.2). Since no statistically significant increases or dose-dependent relationship were observed in micro-nucleated polychromatic erythrocytes, the study author concluded that the result of the study was negative. However, no data demonstrating bone-marrow exposure were presented and, therefore, the negative result of the study was considered by the DS to be of low reliability, also in consideration of the strong increase (4-fold) in the incidence of micro-nucleated polychromatic erythrocytes observed in the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007). In the first study, resulted positive, the mouse strain B6D2F1 was used, while in the new (negative) study a different mouse strain, Crl:CD-1 (ICR), was used, giving reason to be concerned about the sensitivity of this second strain.

In connection with this analysis it is relevant to mention that the applicant is of the opinion that the result of the first micronucleus study (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007) should not be concluded to be positive, since the increase in micronuclei might be within the range of historical negative control values. The view of the RMS for the Pesticide Peer Review was that the concurrent negative control group provided the strongest weight of evidence in comparison with the results from the exposed groups. In studies where a small increase was observed in one exposed group only, i.e. the result was not reproducible; it would be relevant to compare the result with historical negative control values to conclude if the observed increase should be considered to have occurred by chance only. However, in the first study (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016), a 4-fold increase in micronuclei was observed both in the experiment using 24 h sampling time and the experiment using 48 h sampling time. Moreover, in both experiments the increases in micronuclei were dose related. This was considered to clearly demonstrate that the observed effect occurred as a result of exposure to thiophanate-methyl and was not a chance finding.

As thiophanate-methyl has been demonstrated to be mutagenic in the bone marrow of mice, with evidence for both aneugenic and [at that time] clastogenic effects, this effect can be suspected to occur also in germ cells. Therefore, an assessment of the potential of thiophanate-methyl to induce mutations in germ cells was considered necessary by the DS.

A dominant lethal test performed as part of a mutagenic, cytogenetic and teratogenic study gave no indication of an increase in chromosome aberrations. Neither was there an indication of treatment-related dominant lethal mutations in male mice. However, the study suffered from many deficiencies including the lack of reporting of individual data and the absence of a positive control to verify appropriate functioning of the test. Thus, based on this result, it was

difficult to exclude that the aneugenic effects of thiophanate-methyl observed in somatic cells would not occur in germ cells and overall the result was considered inconclusive. Moreover, although the dominant lethal assay is recommended as a method to follow up positive results observed in *in vivo* genotoxicity tests in somatic cells, the DS considered that the test is generally regarded to have low sensitivity and this is also recognised in the OECD TG 478. Furthermore, it is noted that carbendazim, which is concluded to be a germ-cell mutagen (currently classified Muta. 1B, see section on classification) was also negative in dominant lethal tests (DAR on carbendazim, 2009).

Additional data were requested by EFSA during the peer review and consisted of four new studies testing mutagenic effects of thiophanate-methyl in germ cells of mice, one spermatogonial chromosome aberration study with a preceding pre-test and a micronucleus study in germ cells with a preceding pre-test. All four studies were concluded to be negative by the study author, since no statistically significant increase or dose-dependent relationship were observed in chromosome aberrations or micronuclei. However, the DS observed that no data demonstrating target-cell exposure were presented and, therefore, the negative results of the studies have low reliability. The reliability can be further questioned by the arguments presented above for the new bone marrow micronucleus test by (RAR Vol 3, B.6.4.2.2 Doc. No. 557-016), i.e. that the strain Crl:CD-1 (ICR) was used and that there is reason to be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl.

Conclusion by the DS:

Thiophanate-methyl is currently classified Muta. 2; H341 based on a translation from the classification established under the Dangerous Substances Directive. In the CLH report the DS concluded that, as the substance is mutagenic (clastogenic and aneugenic) *in vivo* in somatic cells (bone marrow), systemically available and detected in gonads of rats and mice, the data were considered to indicate "the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells" and classification of thiophanate-methyl in Muta. 1B; H340 was proposed. However, taking into account the additional data submitted in PC, the DS concludes that the available evidence for a clastogenic effect of thiophanate-methyl is weak and, therefore, classification in Muta. 2 for clastogenicity is not proposed. However, based on the positive result of the bone marrow micronucleus study in B6D2F1/Crl mice and the data that resulted in the existing harmonised classification of thiophanate-methyl, the DS considers the classification in Muta 2 for aneugenicity to be appropriate.

Comments received during public consultation

Six comments in total were received during public consultation. Two MSCAs and four companies (an industry consultant, two manufacturers and one downstream user) commented. The MSCAs supported the assessments made by the DS and the conclusion to classify thiophanate-methyl as Muta. 1B; H340 even if with some doubts. The principal argument to support this classification is the fact carbendazim (classified Muta. 1B) is a major metabolites of thiophanate-methyl. The Industry disagreed with the proposed classification and submitted new information in public consultation (see Additional Key Elements section). In summary, industry is of the opinion that based on the complete available data package and the WoE, thiophanate-methyl should not be subject to classification for genotoxicity; the available data provide only a weak evidence for aneugenicity confined to effects seen in somatic cells, while

new and reliable *in vivo* genotoxicity studies on germ cells demonstrated a lack of genotoxicity. Then a classification as Muta 1B is not acceptable for Industry. Details about the argumentation sustained by Industry are in the following section.

Additional key elements

Industry submitted two new toxicokinetic studies in PC (summarized in Table 2 below) in which thiophanate-methyl was administered orally to mice by single administration. One of the studies was performed in B6D2F1/Crl mice and the other was performed in Crl:CD-1 (ICR) mice. In both studies, similar results were obtained demonstrating exposure of plasma and testes to thiophanate-methyl and its metabolites. The concentrations of thiophanate-methyl were comparable in plasma and testes, while the concentrations of metabolites, especially carbendazim, in testes where lower than those in plasma. These studies suggest that the systemic exposure is similar in B6D2F1/Crl mice and Crl:CD-1 (ICR) mice and that toxicokinetic differences could not explain a possible difference in sensitivity between B6D2F1/Crl mice and Crl:CD-1 (ICR) mice when used for detecting genotoxic effects of thiophanate-methyl. However, it is generally considered that differences in toxic effects between strains of a species are only partly explained by toxicokinetic factors. The influence of toxicodynamic factors is commonly believed to be of equal significance, but no information on possible influence of toxicodynamic factors are available from these studies.

Table: *Toxicokinetic data*

Test substance Route Duration of study	Species Strain Sex No./group Vehicle	Dose levels	Main findings	Reference
thiophanate-methyl TK study OECD TG 417 By gavage	ICR male mice 1, 6 and 24 hours after dosing 3 animals for each time point.	2 000 mg/Kg single dose	TM and its metabolites 5-OH-MBC, 4-OH-TM and MBC were detected in the plasma and testis samples collected after 1, 6 and 24h of thiophanate-methyl treatment. Systemic exposure and distribution to the testis of those substances were demonstrated.	Kuroiwa., 2017
thiophanate-methyl TK study OECD TG 417 By gavage	B6D2F1 male mice 1, 6 and 24 hours after dosing 3 animals for each time point.	2 000 mg/Kg single dose	Thiophanate-methyl and its metabolites 5-OH-MBC, 4-OH-TM and MBC were detected in the plasma and testis samples collected after dosing of thiophanate-methyl. Therefore, systemic exposure and distribution to the testis of those substances were demonstrated.	Kuroiwa., 2018

thiophanate-methyl, 5-OH-MBC, 4-OH-TM, MBC analytical reports for validation TK studies by LC/MS/MS method	Plasma and testis of B6D2F1 mouse by LC/MS/MS method	10.0 ug/mL		Akai., 2017; Akai., 2018

In Table 3 the new studies submitted by industry and by a consulting company during PC, are reported. In these studies no increase of structural chromosome aberration was observed therefore the DS concludes that the results of these studies do not support that thiophanate-methyl has clastogenic properties. In addition, due to the negative results in the spermatogonial chromosomal aberration study in Crl:CD-1 (ICR) mice (RAR Vol 3 B.6.4.3.2 Doc. No. RD-03956 (557-012)) and the micronucleus test in germ cells in Crl:CD-1 (ICR) mice (RAR Vol 3 B.6.4.3.3 No. RD-10093 (557-017)), the DS considered that classification in Muta 1B would no longer be warranted.

The DS also noted that the testing conditions varied between studies performed on B6D2F1/Crl and on Crl:CD-1 (ICR) mice. In particular in the positive bone marrow micronucleus study in B6D2F1/Crl mice one treatment was used, while two treatments were used in the negative micronucleus study in Crl:CD-1 (ICR) mice. It is possible that repeated treatments result in a toxicokinetic behaviour that differs from that following single dosing. The available data does not allow for any firm conclusions on the behaviour of thiophanate-methyl in mice during high repeated dosing.

To convincingly demonstrate whether there is a difference in sensitivity or not between the two mouse strains regarding induction of structural and numerical chromosome aberrations, new *in vivo* studies would be required. However the DS considers this request inopportune for ethical reasons and deems that it is preferable to conclude on the basis of the available data, taking the uncertainties described above into consideration.

Overall, the DS concludes that the available evidence for a clastogenic effect of thiophanate-methyl is weak and, therefore, classification in Muta 2 for clastogenicity is not proposed. However, based on the positive result of the bone marrow micronucleus study in B6D2F1/Crl mice and the data that resulted in the existing harmonised classification of thiophanate-methyl, the DS considers the classification in Muta 2 for aneugenicity to be appropriate.

Table: *Additional information on genotoxicity*

Test substance Route Duration of study	**Species Strain Sex No./group Vehicle**	**Dose levels**	**Main findings**	**Reference**
thiophanate-methyl Mammalian bone marrow chromosome aberration	B6D2F1 mice	2 000 mg/kg bw once orally by	Negative. Thiophanate-methyl did not	Aoto., 2018

test OECD TG 475	pre-test	gavage	induce any structural and/or numerical chromosomal aberrations in mouse bone marrow at the limit dose level defined in the OECD TG 475. There was no increase of bone marrow cells with chromosomal aberration (including polyploidy) in thiophanate-methyl. 2 000 mg/kg administered group at each sampling time.	
thiophanate-methyl Mammalian Bone Marrow Chromosomal Aberration OECD TG 475 B6D2F1 mice main test	B6D2F1 mice Six male mice for group	0, 500, 1 000 or 2 000 mg/kg bw once orally by gavage	Negative No increase of bone marrow cells with structural and/or numerical chromosomal in this strain of mice. aberration by thiophanate-methyl treatment	Kuboki 2018 RD-10440
6 different chemicals: -colchicine, -7,12-dimcthylbenz[a]anthracene, -ethylmethanesulfonate; -V-ethyl-V-nitrosourea, -6-mercaptopurine,	Male mice of 4 strains Sic: ddY (ddY); CRJ: CD-l(ICR) (CD-1); Sic: BDF, (BDF)		All 4 strains gave positive results with all 6 chemicals, although ms tended to show the highest responses. ddY and CD-1 were low responders. While BDF, was	CSGMT, 1988

-potassium Chromate, The Collaborative Study Group for the Micronucleus Test (CSGMT)	ms: Hai (ms)		intermediate between ms and the other two	
Three benzimidazoles: -benomyl, -methyl thiophanate -methyl 2-benzimidazole carbamate (MBC), MN in bone marrow structural chromosome aberrations plus or minus gaps, aneugenic effects (hyperdiploidy or polyploidy)	Male Swiss Albino mice Single p.o.		Positive Methylthiophanate significantly induced micronuclei, but it was less effective than benomyl and MBC	Barale *et al.*, 1993
thiophanate-methyl micronucleus test, comet assay chromosome analysis.	lizard *Podarcis sicula* (*Reptilia, Lacertidae*)	four groups Group A , control animals (9 M/9 F) sprayed twice weekly with 100 mL of water; and 3 Groups of exposed sprayed twice weekly with 100 mL of 1.5 % thiophanate-methyl (1.5 g thiophanate-methyl in 100 mL of water, the concentration sprayed on fruit crops and	Positive thiophanate-methyl exposure induces genomic damage (measured as MN induction, comet tail length and chromosome aberrations) and the frequency of these markers and the exposure time are significantly correlated.	Capriglione *et al.*, 2011

		ornamental plants);		
Mutagenic, Cytogenetic and Teratogenic Studies on thiophanate-methyl.	ICR-mice male (for mutagenicity) and Wistar strain albino male rats (for cytogenicity)	Dose for mutagenicity 8 40 200 400 500 mg/kg bw Dose for cytotoxicity 1 000, 500 200 and 40 mg/kg bw/d	In the dominant lethal mutation experiment, administration of a single dose of thiophanate-methyl to adult male mice produced no distinct mutagenic effects. Thiophanate-methyl was not capable of inflicting cytogenetic damage to somatic meiotic chromosomes of the rats.	Makita *et al.*, 1973
Bone marrow micronucleus assay: a review of the mouse stocks used and their published mean spontaneous micronucleus frequencies.				Salamone, *et al.*, 1994

Assessment and comparison with the classification criteria

At present, thiophanate-methyl is classified as Muta. 2; H341 based on a translation of the classification Muta. Cat. 3 established under Council Directive 67/548/EEC, now replaced by Regulation (EC) No 1272/2008. Carbendazim, a metabolite of thiophanate-methyl, is classified as Muta. 1B; H340.

In the absence of human data, the criteria for classification in category 1A are not met.

In the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017) the following is stated: "*It could be argued that in a case where in vivo mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, then that substance should also be considered as a Category 1B mutagen. Germ cell mutagens as the spermatogonia are generally not protected from substance exposure by the blood-testes barrier formed by the Sertoli cells. In such circumstances the relevant criteria are as follows:*

-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".

With respect to the above it is of importance to note that, according to data in the section on toxicokinetics (Vol 3, section B.6.1.1.1 of RAR), levels of labelled parental thiophanate-methyl comparable to residue levels in the blood are found in testis and ovaries of rats administered a single dose of the compound. In rats administered repeated doses of the substance the levels in ovaries were initially higher (at 3 hours) than the levels in blood. Exposure of testis was also demonstrated in mice receiving a single dose of thiophanate-methyl. New studies were provided by Industry in the Public Consultation in order to evaluate the systemic exposure and target organ accessibility of the test substance in two different mice strains ICR and B6D2F1 in single-dose oral toxicokinetic studies. In both strains comparable concentrations of thiophanate-methyl were found in plasma and testis and the concentrations of metabolites, in particular Carbendazim, in the testis were lower than those in the plasma (Kuroiwa Y, 2017; Kuroiwa Y, 2018).

Overall, this indicates that gonads are exposed to the substance and its metabolites. Moreover, according to data in the section on toxicokinetics, urinary metabolites carbendazim and 5-OH-carbendazim-S are formed to an extent of ≤ 1 % and 42 % of the total administered radioactivity respectively.

Considerations on structure-activity relationship

Carbendazim, a metabolite of thiophanate-methyl, is characterised by the benzimidazole moiety, which is known to be associated with aneugenic activity, based on its ability to bind tubulin and consequently inhibit its polymerisation(1).

On the other hand, the presence of the electrophilic carbamate moiety in both thiophanate-methyl and carbendazim points to a possible DNA-reactive mechanism (2).

However, the experimental results suggest that in the case of thiophanate-methyl the aneugenic mechanism appears to be prevalent while a contribution of clastogenicity to the mutagenicity of the substance is not demonstrated (See Friedman, P. A. and Platzer, E. G., 1978 and Benigni and Bossa, 2011).

Considerations on threshold

For thiophanate-methyl the threshold for aneuploidy in cultured human peripheral blood lymphocytes was 6 µg/mL and the no effect level was 4 µg/mL (Marshall, 1997). For carbendazim, the threshold concentrations for aneuploidy were determined in two independent studies in cultured human peripheral blood lymphocytes. Elhajouji and co-workers reported ~200 ng/mL and ~500 ng/mL for non-disjunction and chromosome loss, respectively (Elhajouji A. *et al*. 1997), while Bentley reported 600 ng/mL for both endpoints (Bentley K. *et al.* 2000).
In a toxicokinetic study in which thiophanate-methyl was administered once orally to B6D2F1/Crl mice at 2 000 mg/kg bw (corresponding to the highest concentration used in the *in vivo* MN study) thiophanate-methyl and carbendazim were detected in testis at concentrations slightly over the threshold for aneugenic effects for both thiophanate-methyl and Carbendazim, as reported in the table below (Koroiwa, 2018).

Table: *Summary of testis concentration of thiophanate-methyl and its metabolite as reported in Koroiwa, 2018*

Modified from Koroiwa, 2018

Dose (mg/kg)	Analyte	Testis concentration (ng/g wet wt) Mean(n=3)		
		1h	6h	24h
2000	Thiophanate-methyl	8690	8690	3840
	5-OH-MBC	247	454	162
	4-OH-TM	BLQ	BLQ	BLQ
	MBC	403	302	262

BLQ : Below limit of quantification (100 ng/g wet wt)
BLQ was calculated as zero (0) for Mean.
NA : Not applicable

Thiophanate-methyl was negative in a spermatogonial chromosomal aberration study in Crl:CD-1 (ICR) mice, (RAR Vol 3 B.6.4.3.2 Doc. No. RD-03956 (557-012)) and in a dominant lethal assay (RAR Vol 3 B.6.4.3.1 Doc. No. RD-73062 (551-006). However, it should be considered that the Spermatogonial assay was performed in the Crl:CD-1 stain, where also the MN assay results were negative. Moreover the spermatogonial assay is designed to detect structural chromosomal aberration but is not able to detect aneugenic effects. The dominant lethal assay is kmown not to be very sensitive and also the recognised aneugen carbendazim was negative in this assay. Thiophanate-methyl was also negative in a micronucleus test in germ cells in Crl:CD-1 (ICR) mice (RAR Vol 3 B.6.4.3.3 No. RD-10093 (557-017), however this MN assay is not validated in germ cells and no OECD TG is available. Moreover the strain used (Crl:CD-1 (ICR)) was negative also in the MN test in erythrocytes (RAR Vol 3 B.6.4.2.2 Doc. No. 557-016) and the response of positive controls was rather weak, indicating a relatively low sensitivity of the test system.

It is noted that the concentrations reached in testis after the oral administration of dosages currently used in the *in vivo* test are close to those found to be effective *in vitro*. On the other hand, the available experimental studies on germ cells did not demonstrate any genotoxic effect, although these study showed methodological limits and shortcoming.

Conclusions

Thiophanate-methyl is currently classified Muta. 2; H341 based on a translation from the classification established under the Dangerous Substances Directive. The QSAR analysis indicates the presence of structural alerts for both aneugenicity and clastogenicity, however the experimental results suggest that for the mutagenicity of thiophanate-methyl the aneugenic mechanism is prevalent while clastogenicity is not demonstrated.

The available experimental studies indicate that thiophanate-methyl is aneugenic *in vitro* and *in vivo* in somatic cells (bone marrow), thus meeting the criteria for classification as a mutagen. RAC however notes that the *in vivo* evidence reported in a mouse strain (B.6.4.2.1) was not confirmed in a different strain (B.6.4.2.2).

The substance is systemically available and was detected in gonads of mice at concentrations close to those found to cause aneugenicity *in vitro*. On the other hand, the available *in vivo* studies in germ cells did not demonstrate any genotoxic effect. Taking into account all available data in a weight of evidence approach, RAC is of the view that criteria for classification in Category 1B are not met.

In conclusion RAC is of the opinion that Thiophanate-methyl should be classified as **Muta. 2; H341 (suspected of causing genetic defects).**

10.9 Carcinogenicity

Table 38: Summary table of animal studies on carcinogenicity

Test substance **Route** **Duration of study**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Dose levels**	**Main findings**	**Reference**
Thiophanate-methyl Oral (dietary) 24 months Acceptable	Rat Fischer-344 60/sex/dose	0, 75, 200, 1200 and 6000 ppm (equivalent to 0, 3.3, 8.8, 54.4 and 280.6 mg/kg bw/day for males and 0, 3.8, 10.2, 63.5 and 334.7 mg/kg bw/day for females.)	1200 ppm: ↓ Bw (9-16%) ↑ Thyroid weights (24% and 27% in males and females, respectively) ↑ Liver weight (25% and 24% in males and females, respectively) ↑ kidney weight (20% and 8% in males and females, respectively) - Morphological and functional changes in the thyroid, kidneys and hepatocellular hypertrophy and occurrence of lipofuscin (indicating a liver cell damage, accelerated nephropathy and lipidosis of the adrenal cortex. ↑ Follicular cell hyperplasia and hypertrophy (M: 38%, p<0.01, F: 47%, p<0.001) ↑ Follicular cell adenomas (M: 7%, not statistically significant, M: 20%, p<0.05) -Haematological changes (↓ red blood cell count, ↓ MCV, ↓ MCH, ↓ MCHC) - Effects on clinical chemistry More pronounced effects at 6000 ppm: ↑ Mortality rate 6000 ppm (53/55 males died, cause of death was mainly accidents, nephropathy, thyroid tumours and leukaemia) ↑ Follicular cell hyperplasia and hypertrophy (M: 97%, F: 98%, p<0.001) ↑ Follicular cell adenomas (20% in males, p<0.01, 3% in females) - ↑ Follicular cell adenocarcinomas (M: 5%, not statistically significant. F: 0%)	RAR Vol 3 B.6.5.1
Thiophanate-methyl Oral (dietary) 24 months Supportive	Rat Sprague-Dawley 35/sex/dose	0, 10, 40, 160 and 640 ppm (equivalent to 0, 0.5, 2, 7 and 30 mg/kg bw/day for males and 0, 0.5, 2, 8 and 34 mg/kg bw/day for females.)	640 ppm: ↓ Bw gain - histopathological changes in the testes (reduction in spermatogenesis) and the thyroid (follicular epithelium hypertrophy, decrease of colloidal substance)	RAR Vol 3 B.6.5.2
Thiophanate-methyl	Mouse	0, 150, 640, 3000,	3000 ppm (m) 640 (f):	RAR Vol 3

(Topsin M*) Oral (dietary) 18 month Acceptable	Crl:CD-1 (ICR)BR 60/sex/dose	and 7000 ppm (equivalent to 0, 23.7, 98.6, 467.6 and 1078.8 mg/kg bw/day for males and 0, 28.7, 123.3, 557.9 and 1329.4 mg/kg bw/day for females)	↓ Bw (11-17%) ↑ Liver weight (24-82%) ↑ Thyroid weight (110%) ↑ Heart weight (f: 23-39%) ↑ Hepatocellular centrilobular hypertrophy ↑ Hepatocellular adenomas (M: 50% of terminal necropsies ($p<0.05$) at 3000 ppm; 69% of terminal necropsies ($p<0.05$) at 7000 ppm. F: 24% of terminal necropsies ($p<0.05$) at 3000 ppm; 59% of terminal necropsies ($p<0.05$) at 7000 ppm)	B.6.5.3
Thiophanate-methyl Oral (dietary) 24 month Supportive	Mouse ICR-SLC 50/sex/dose	0, 10, 40, 160, and 640 ppm (equivalent to 0, 1.2, 4.4, 20 and 82 mg/kg bw/day for males and 0, 1.3, 5.0, 19 and 82 mg/kg bw/day for females)	640 ppm: ↓ Bw gain (10%) No increase in the incidence of neoplasia at any dose level.	RAR Vol 3 B.6.5.4
Thiophanate-methyl Oral (dietary) 24 month Supportive	Beagle dogs 5/sex/dose, 4/sex/dose at highest dose.	to 0, 2, 19, 50 and 250 mg/kg bw/day in capsules	250 mg/kg bw/day: Slight retardation of growth in both sexes. A moderate hypertrophy of the thyroid was noted in males and females at 50 and 250 mg/kg bw/d based on organ weight and histopathological examination.	RAR Vol 3 B.6.5.5

* The test substance was referred to as "Topsin M". According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 96.6 and 95.9 % in the two batches used in the study.

Table 39: Summary table of human data on carcinogenicity

No data.

Table 40: Summary table of other studies relevant for carcinogenicity

No data.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

The data available to assess this endpoint include five long-term studies. These are thoroughly described in Annex I (Volume 3 to the RAR).

Two of the studies are considered acceptable for classification purposes whereas the remaining three are regarded as supportive information only. All studies were thoroughly assessed in the first DAR of 1997. For this assessment, the studies considered acceptable are presented in detail whereas the studies considered supportive are only briefly described. In rats, an increased incidence of thyroid follicular cell hypertrophy and hyperplasia as well as an increased incidence of thyroid follicular cell adenomas were noted.

Table 41: Thyroid changes

	Incidence of thyroid follicular cell (FC) changes (%)				
Males	**0 ppm**	**75ppm**	**200 ppm**	**1200 ppm**	**6000 ppm**
Diffuse FC hyperplasia/ hypertrophy	0/60 (0)	0/58 (0)	0/60 (0)	23/60 (38)**	58/60 (97)***
Focal FC hyperplasia	3/60 (5)	2/58(3)	2/60 (3)	3/60 (5)	15/60 (25)**
FC adenoma	1/60 (2)	0/58 (0)	0/60 (0)	4/60 (7)	12/60 (20)**
FC adenocarcinoma	0/60 (0)	0/60 (0)	0/60 (0)	0/60 (0)	3/60 (5)
Females	**0 ppm**	**75 ppm**	**200 ppm**	**1200 ppm**	**6000 ppm**
Diffuse FC hyperplasia/ hypertrophy	1/60 (2)	1/59 (2)	0/60 (0)	28/60 (47)***	59/60 (98)***
Focal FC hyperplasia	0/60 (0)	1/59 (2)	0/60 (0)	4/60 (7)	8/60 (13)*
FC adenoma	0/60 (0)	0/59 (0)	0/60 (0)	1/60 (2)	2/60 (3)
FC adenocarcinoma	0/60 (0)	0/59 (0)	0/60 (0)	0/60 (0)	0/60 (0)

*p<0.05, **p<0.01, ***p<0.001

Mortality was high in the high dose-males as 53/55 of them died on study. 8 of these were killed in extremis at weeks 11 and 12 as they showed a fracture of the nasal bone and subsequent dyspnoea (rhinorrhagia) which was attributed to the feeding stations (not test substance related). The main causes of death noted in males of the same dose group were nephropathy (22 rats), thyroid follicular cell tumours (10 rats) and leukaemia (6 rats). The severe nephropathy was associated with hyperplasia of the parathyroid, demineralization of the bone and metastatic calcification in various organs. It should be noted that leukaemia is common in the F344 rat. At this dose level, the MTD was exceeded.

The adenomas were considered related to treatment and assumed to result from effects on thyroid hormone production or release, a conclusion based on the results from a mechanistic study including the following experiments:

• Measurement of T3, T4 and TSH, drug intake, liver and thyroid weights and total cholesterol in serum. Measurements of enzyme induction: cytochrome P450, cytochrome b5, NADPH-cytochrome c reductase, UDP-glucuronosyltransferase) and microsomal protein (rat)

• Additional administration of thiophanate-methyl with daily T4 supplementation, measurements of body weight, thyroid and liver weights and total cholesterol levels (rat)

• Peroxidase activity measurements (pig) using PTU as a reference substance

• Proliferation of liver cells (mice and rats) using PB as a reference substance

As the increase of thyroid follicular cell adenomas was only statistically significant in high dose males and the dose seems to have been above the maximum tolerated dose, the tumours are not considered to demonstrate a carcinogenic potential of thiophanate-methyl.

There were no thyroid tumours noted in mice but a statistically significant increase of hepatocellular adenomas was observed in animals males and females administered dietary concentrations of >3000 ppm thiophanate-methyl. In addition, one hepatocellular carcinoma was observed in the second lowest dose and in the top dose male group. In the high dose group, there was a concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis which is not considered to be test-substance related and the MTD is therefore not considered to have been reached in this study.

The previous evaluation considered this effect the tumours to result from the induction of the cytochrome P450 drug metabolising system, an effect known to occur in mice following exposure to xenobiotics. This conclusion, which was shared by the study author, was based on the results from the mechanistic study showing an increased liver weight and increased proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. However, for this review, this data is not considered to demonstrate that the hepatocellular adenomas observed arise due to a phenobarbital-like mode of action lacking human relevance (see section 10.12).

Table 42: Incidence of mouse hepatocellular adenomas.

	Incidence of hepatocellular adenomas (%)				
Dose level (ppm)	**0**	**150**	**640**	**3000**	**7000**
Males					
Unscheduled necropsies	0/10 (0)	0/11 (0)	0/14 (0)	2/16 (12.5)	6/24 (25)
Terminal necropsies	4/40 (10)	8/39 (20.5)	7/36 (19.4)	17/34 (50) **	18/26 (69.2)**
Combined	4/50 (8)	8/50 (16)	7/50 (14)	19/50 (38)**	24/50 (48)**
Females					
Unscheduled necropsies	0/12 (0)	0/13 (0)	0/15 (0)	0/17 (0)	2/23 (8)
Terminal necropsies	0/38 (0)	0/37 (0)	3/35 (8.6)	8/33 (24.2) **	16/27 (59.3) **
Combined	0/50 (0)	0/50 (0)	3/50 (6)[a]	8/50 (16)**	18/50 (36)**

**p<0.01

[a] outside historical control range (HCD: Males: 0-16.3% (mean 8.2%); Females: 0-2.7% (mean 1.4%))

10.9.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), substances are classified for carcinogenicity in **Category 1** (known or presumed human carcinogens) on the basis of epidemiological and/or animal data. Category 1 is subcategorised into 1A if the substance is "*known to have carcinogenic potential for humans, classification is largely based on human evidence*" and 1B if "*presumed to have carcinogenic potential for humans, classification is largely based on animal evidence*."

As there is no human data available for thiophanate-methyl that may be relevant for carcinogenicity, criteria for category 1A are not fulfilled.

For classification in category 1B evidence may be derived from "*[..] animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen) [..] In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals*."

Sufficient evidence from animal studies is explained as *"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. [..]"*

The placing of a substance in **Category 2** (suspected human carcinogens) "*is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies*."

Limited evidence from animal studies is explained as "*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*."

The significance of tumours observed in the chronic and carcinogenicity studies, *i.e.* thyroid follicular cell adenomas in rats and hepatocellular adenomas in mice is discussed below based on considerations included in the CLP guidance:

(a) tumour type and background incidence;

Rats: thyroid follicular cell adenomas. Three cases of adenocarcinoma in the highest dose group. The study report does not include historical control data.

Mice: hepatocellular adenomas. According to information stated in the study report, the historical control incidence of hepatocellular adenoma compiled by Charles River Laboratories for their CD-mice on 18-month studies for the time period 1978-1984s are[3]:

Males: 0-16.3% (mean 8.2%)

Females: 0-2.7% (mean 1.4%)

For WIL Research Laboratories, Inc., the incidences recorded in the "most recent" 18-month carcinogenicity study were 16.4% (9/55) and 1.8% (1/55) in males and females respectively. The frequencies observed in the study are outside of the background incidence and the tumours can thus be considered to result from treatment with thiophanate-methyl.

(b) multi-site responses;

Rats: The only tumour type observed at higher frequency than in controls was thyroid follicular cell adenoma in males.

Mice: The only tumour type observed at higher frequency than in controls was hepatocellular adenoma.

(c) progression of lesions to malignancy;

Rats: Follicular cell adenocarcinomas were observed in 3/60 high dose males compared to none in controls. This may indicate a progression into malignancy.

Mice: Hepatocarcinoma was observed in one male of the high dose group (7000 ppm) and in one male of the second lowest (640 ppm) dose group. Considering the lack of a dose-response, this is not considered to indicate progression into malignancy.

(d) reduced tumour latency;

Rats: Follicular cell hyperplasia and hypertrophy were noted in both sexes from the two highest dose groups both at interim sacrifice (12 months) and at terminal sacrifice (24 months). Follicular cell adenocarcinomas were only observed at terminal sacrifice indicating slow tumour development.

Mice: At interim sacrifice (9 months), an increase in hepatocellular centrilobular hypertrophy was observed in mice from the two highest treatment groups and mid-dose females. Moreover, a higher severity grade was observed in high dose animals and in the males of the next highest treatment group. Hepatocellular adenomas were only observed at terminal sacrifice indicating slow tumour development.

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

Rats and mice: The tumour types observed in rats and mice occurred in both sexes but the increase of thyroid follicular cell adenomas was only statistically significant in high dose male rats.

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

Follicular cell adenocarcinomas and hepatocellular adenomas were observed in rats and mice, respectively.

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

No information available.

[3] Spontaneous Neoplastic Lesions in the Crl:CD-1®(ICR)BR Mouse, Charles River Laboratories, Inc.

(h) routes of exposure;

Information restricted to studies performed using oral administration (via diet).

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

A comparative in vitro metabolism study is available (RAR Vol 3, B.6.1.4) and showed no differences in metabolism between humans and rats.

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

Rats: There was an increase in mortality in the top dose group which was partially linked to the thyroid tumours. Although the high rate of mortality could, at least in part, be due to excessive toxicity, the treatment relationship of thyroid tumours was considered real. Follicular cell ademonas of the thyroid were only statistically significant in animals administered the highest dietary concentration (6000 ppm). Adenocarcinomas were also only observed at this dose level.Mortality was high at 6000 ppm as 53 of 55 males died. 8 of these were killed in extremis at weeks 11 and 12 as they showed a fracture of the nasal bone and subsequent dyspnoea (rhinorrhagia) which was attributed to the feeding stations (not test substance related). The main causes of death noted in males of the same dose group were nephropathy (22 rats), thyroid follicular cell tumours (10 rats) and leukaemia (6 rats). The severe nephropathy was associated with hyperplasia of the parathyroid, demineralization of the bone and metastatic calcification in various organs. It should be noted that leukaemia is common in the F344 rat. At this dose level, the MTD was exceeded.

Mice: Hepatocellular adenomas occurred in all treated animals but the frequencies were only statistically significant in the two highest treatment groups (3000 and 7000 ppm). In the high dose group, there was a concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis which is not considered to be test-substance related and the MTD is therefore not considered to have been reached in this study.

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

The substance induces aneuploidy and clastogenicity in somatic cells and could thus be suspected to have a carcinogenic potential at levels above a threshold level. However, since only a single tumour type was observed in rats and mice there are no clear indications of a link between genetic instability and cancer.

Rats: Increased thyroid weight with hypertrophy/hyperplasia and sometimes accompanying effects on thyroid hormones were observed in many of the repeated dose toxicity studies available (including studies on short-term toxicity, long-term toxicity reproduction toxicity and neurotoxicity). In many of the studies there was also effects on the liver which could possibly indicate an up-regulation of the thyroid in response to increased hepatic clearance of T4 by uridine diphospho-glucuronosyltransferase (UDPGT). This is a rodent-specific effect generally considered to lack relevance for humans. Data from the mechanistic study indicate that the hypertrophy of the thyroid and the TSH response is counteracted by T4 supplementation supporting effects being due to a negative feedback mechanism. Data also indicate induction of cytochrome P450 and related drug metabolising enzymes including an increase of UDPGT in the high dose group. However, since T4 supplementation did not influence liver weight and since the pattern differed from phenobarbital, the increased UDPGT does not seem to be the sole explanation for the thyroid effects observed. The results indicated an inhibition of thyroid peroxidase in swine thyroids and this seems to be the principal reason for the T4 depression (see section 10.12-2). Therefore, it is not considered safe to exclude that the thyroid effects observed could be relevant for humans.

Mice: Data in the mechanistic study suggest that hepatocellular adenomas result from induction of the cytochrome P450 drug metabolising system. However, the data available to support this claim is limited to an increased liver weight and proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. This is not considered to conclusively link tumours to a phenobarbital-like mode of action lacking human relevance.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Life-time exposure to thiophanate-methyl resulted in an increased frequency of thyroid adenomas in rats and hepatocellular adenomas in mice. Human relevance cannot be excluded; however, the tumour types are mainly benign. MTD seems to have been met in the rat study but not in the mouse study. Overall, data is considered as “limited” evidence of carcinogenicity and classification in **Carc. 2 H351** is proposed for thiophanate-methyl. for thiophanate-methyl.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The data available to assess this endpoint include five long-term studies.
Two of the studies are considered acceptable for classification purposes whereas the remaining three are regarded as supportive information only.

In the first key study (B.6.5.1 in the RAR), conducted in rats, an increased incidence of thyroid follicular cell hypertrophy and hyperplasia as well as an increased incidence of thyroid follicular cell adenomas were noted.

Table: *Thyroid changes*

Incidence of thyroid follicular cell (FC) changes (%)					
Males	**0 ppm**	**75ppm**	**200 ppm**	**1 200 ppm**	**6 000 ppm**
Diffuse FC hyperplasia/ hypertrophy	0/60 (0)	0/58 (0)	0/60 (0)	23/60 (38)**	58/60 (97)***
Focal FC hyperplasia	3/60 (5)	2/58(3)	2/60 (3)	3/60 (5)	15/60 (25)**
FC adenoma	1/60 (2)	0/58 (0)	0/60 (0)	4/60 (7)	12/60 (20)**
FC adenocarcinoma	0/60 (0)	0/60 (0)	0/60 (0)	0/60 (0)	3/60 (5)
Females	**0 ppm**	**75 ppm**	**200 ppm**	**1 200 ppm**	**6 000 ppm**
Diffuse FC hyperplasia/ hypertrophy	1/60 (2)	1/59 (2)	0/60 (0)	28/60 (47)***	59/60 (98)***
Focal FC hyperplasia	0/60 (0)	1/59 (2)	0/60 (0)	4/60 (7)	8/60 (13)*
FC adenoma	0/60 (0)	0/59 (0)	0/60 (0)	1/60 (2)	2/60 (3)
FC adenocarcinoma	0/60 (0)	0/59 (0)	0/60 (0)	0/60 (0)	0/60 (0)

*p < 0.05, **p < 0.01, ***p < 0.001

The MTD was exceeded in the male group at the top dosage, where 53/55 animals died during the study. 8 of these were killed in extremis at weeks 11 and 12 as they showed a fracture of the nasal bone and subsequent dyspnoea (rhinorrhagia) which was attributed to the feeding stations and considered not test substance related. The main causes of death noted this group were nephropathy (22 rats), thyroid follicular cell tumours (10 rats) and leukaemia (6 rats). The severe nephropathy was associated with hyperplasia of the parathyroid, demineralization of the bone and metastatic calcification in various organs. It should be noted that leukemia is common in the F344 rat.

The adenomas were considered related to treatment and assumed to result from effects on thyroid hormone production or release, a conclusion based on the results from mechanistic studies including the following experiments:

- Measurement of T3, T4 and TSH, drug intake, liver and thyroid weights and total cholesterol in serum. Measurements of enzyme induction: cytochrome P450, cytochrome b5, NADPH-cytochrome c reductase, UDP-glucuronosyltransferase) and microsomal protein (rat).
- Administration of thiophanate-methyl followed by daily T4 supplementation, measurements of body weight, thyroid and liver weights and total cholesterol levels (rat).
- Thyroid peroxidase (TPO) activity measurements (pig) using PTU as a reference

substance

- Proliferation of liver cells (mice and rats) using PB as a reference substance.

As the increase of thyroid follicular cell adenomas was only statistically significant in high dose males and the dose seems to have been above the maximum tolerated dose, the tumours are not considered to demonstrate a carcinogenic potential of thiophanate-methyl.

In the second key study, conducted in mice (Table 6), no thyroid tumours were reported but a statistically significant increase of hepatocellular adenomas was observed in males and females administered dietary concentrations of 3 000 and 7 000 ppm thiophanate-methyl.

Table: *Incidence of mouse hepatocellular adenomas*

Incidence of hepatocellular adenomas (%)					
Dose level (ppm)	**0**	**150**	**640**	**3 000**	**7 000**
Males					
Unscheduled necropsies	0/10 (0)	0/11 (0)	0/14 (0)	2/16 (12.5)	6/24 (25)
Terminal necropsies	4/40 (10)	8/39 (20.5)	7/36 (19.4)	17/34 (50) **	18/26 (69.2)**
Combined	4/50 (8)	8/50 (16)	7/50 (14)	19/50 (38)**	24/50 (48)**
Females					
Unscheduled necropsies	0/12 (0)	0/13 (0)	0/15 (0)	0/17 (0)	2/23 (8)
Terminal necropsies	0/38 (0)	0/37 (0)	3/35 (8.6)	8/33 (24.2) **	16/27 (59.3) **
Combined	0/50 (0)	0/50 (0)	3/50 (6)a	8/50 (16)**	18/50 (36)**

**p < 0.01

a outside historical control range (HCD: Males: 0-16.3 % (mean 8.2 %); Females: 0-2.7 % (mean 1.4 %))

In addition, one hepatocellular carcinoma was observed at 640 ppm and at 7 000 ppm dosage in the male group. In the 7 000 ppm dose group, there was a concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis which is not considered to be test-substance related and the MTD is therefore not considered to have been reached in this study.

The previous evaluation considered this effect the tumours to result from the induction of the cytochrome P450 drug metabolising system, an effect known to occur in mice following exposure to xenobiotics. This conclusion, which was shared by the study author, was based on the results from the mechanistic study showing an increased liver weight and increased proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. However, these data are not considered by the DS to demonstrate that the hepatocellular adenomas observed arise due to a phenobarbital-like mode of action lacking human relevance (see section 10.12 of the CLH report and the STOT RE section in this document).

Other information to support the carcinogenicity relevance to human are the following:

1. Long-term toxicity and carcinogenicity study in rats (B6.5.2 in the RAR). In this study the number of parameters examined (clinical chemistry and haematological examination) was not as extensive as required by the OECD TG 452, but it is considered to provide useful supportive information on the toxicological profile of the test substance. In particular histopathological changes in the thyroid (follicular epithelium hypertrophy, decrease of colloidal substance) and in the testes (reduction in spermatogenesis) were reported. It is also noted that the dose range used was lower than in the study reported above (B.6.5.1 in the RAR).

2. Long-term toxicity and carcinogenicity study in Beagle dogs 24 months (B.6.5.5 in the RAR) where some deviations from OECD TG 452 (version 1982). A moderate hypertrophy of the thyroid was noted in males and females at 50 and 250 mg/kg bw/d based on organ weight and histopathological examination. No effects on PBI (Protein binding iodine) levels were reported. Although no effect on carcinogenesis was observed, it is noted that the duration of the study (24 months) if compared to the lifespan of the dog was much shorter than the duration of the rodent study.

3. Chronic (1 year) oral toxicity study in the dog via capsule administration with thiophanate-methyl (B.6.3.2.5 in the RAR). Increased thyroid weight in females at lowest dose and at higher dose level a decrease of T4 in males and histopathological changes in the thyroid (hypertrophy and hyperplasia) were observed. No effect on carcinogenicity was reported. As noted for the study above, the duration of the study was not comparable to those applied to rodents.

The DS concludes that life-time exposure to thiophanate-methyl resulted in an increased frequency of thyroid adenomas in rats and hepatocellular adenomas in mice. Human relevance cannot be excluded; however, the tumour types are mainly benign. MTD seems to have been exceeded in the rat study but not in the mouse study. Overall, the data are considered as "limited" evidence of carcinogenicity. Taking into account the comments received in public consultation, the DS considered that at the highest dose level in the 2-year rat study, where the statistically significant increase in adenomas were noted, the MTD was probably exceeded. As at the dose below the increase of adenoma frequency was not statistically significant the DS concluded that the results of this study no longer justify the classification as Carc. 2 H351, as originally proposed in the CLH report.

Comments received during public consultation

Several comments were received in public consultation. In particular, three MS supported the originally proposed classification as carcinogenicity cat 2 because also in line with the opinion expressed in the pesticide peer review process (EFSA 2018, doi: 10.2903/j.efsa.2018.5133, page 10). Moreover, one MS recalled that the substance has a structural alert for non-genotoxic carcinogenicity (thiocarbonyl) when put through the OECD toolbox and that there is a publicly available EPA report with information on the carcinogenicity of thiocarbonyl compounds. In this report the type of carcinogenicity observed is similar (predominantly thyroid hypertrophy/hyperplasia by inhibiting thyroid hormones resulting in tumours, but also liver tumours in mice as also observed for thiophanate-methyl), suggesting a possible similar carcinogenic mechanism. Two industries commented the carcinogenicity in PC, both expressing disagreement with the proposed classification. The principal elements discussed are: i) the rats tumours are

observed beyond the MTD and ii) tumours observed in mice and rats are not relevant for humans. The DS agreed with industry for the first point, but disagreed with the argumentation regarding relevance to humans, where the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are considered secondary to liver enzyme induction and not relevant to humans. New mechanistic studies were provided by industry in PC where the thyroid peroxidase (TPO) inhibition demonstrated a lack of relevant inhibition for human TPO *in vitro* when tested up to precipitating concentrations.

Additional key elements

The new data provided by Industry during public consultation are summarized in the following table:

Table: *In vitro studies for Thyroid Peroxidase Inhibition (TPO)*

Test substance Route Duration of study	**Species Strain Sex No./group Vehicle**	**Dose levels**	**Main findings**	**Reference**
thiophanate-methyl IN-VITRO assay Guaiacol assay of TPO activity. No GL available	Dog Microsomes prepared from non-juvenile female Beagle dogs thyroids.	TPM: 0.1 to 500 µM, 11concentrations TPU = positive control 0.01 to 100 µM, 9 concentrations;	thiophanate-methyl induced a weak partial TPO inhibition *in vitro* in dog thyroid microsomes when tested up to its solubility limit in the Guaiacol assay of TPO activity. Referring to *in vivo* conditions, thiophanate-methyl is considered not to be a TPO inhibitor in the dog.	Haines C., 2018 RD 10598
thiophanate-methyl IN-VITRO	Human Microsomes prepared from	TPM: 0.1 to 500 µM, 11concentrations TPU=positive control 0.01 to 100 µM, 9	thiophanate-methyl did not cause any appreciable	Haines C., 2018 RD 10588

assay Guaiacol assay of TPO activity. No GL available	non-juvenile female human thyroperoxidase	concentrations;	inhibition of TPO activity at concentrations up to its solubility limit.	
thiophanate-methyl *In vitro* assay Guaiacol assay of TPO activity. No GL available	Pig Microsomes prepared from non-juvenile female Yorkshire pig thyroperoxidase	thiophanate-methyl and TPU=positive control 0.01 to 100 µM, 9 concentrations/substance	thiophanate-methyl not cause any appreciable inhibition of TPO activity at concentrations up to its solubility limit. thiophanate-methyl is not an inhibitor of porcine TPO in this assay.	Haines C., 2018 RD 10590
thiophanate-methyl *In vitro* assay Guaiacol assay of TPO activity. No GL available	Rat Microsomes prepared from non-juvenile female Han Wistar rat thyroids	thiophanate-methyl and TPU=positive control 0.01 to 100 µM, 9 concentrations/substance	thiophanate-methyl induced a weak partial TPO inhibition *in vitro* in rat thyroid microsomes when tested up to its solubility limit. thiophanate-methyl is considered not to be a TPO inhibitor in the rat.	Haines C., 2018 RD 10591
TPO activity inhibition assay thyroids was investigated	human microsomes prepared from female human thyroids	thiophanate-methyl: 0.1 to 500 µM, 11 concentrations TPU=positive control 0.01 to 200 µM,	thiophanate-methyl did not cause any appreciable inhibition of TPO activity at	Haines C., 2018 tier 2 summaries RD 10588

		10 concentrations	concentrations up to its solubility limit.	

All new *in vitro* studies showed no induction of TPO neither in pig, rats, mice or human. The DS states that this result is in contrast to the result observed in the mechanistic study reported in the CLH where an inhibition of TPO in pig was observed. In this regard RAC observed that in the experiment conducted in microsomes prepared from pig (Haines C., 2018) the solubility limit concentration was not reached therefore a firm conclusion cannot be drawn in this species.

The additional mechanistic studies submitted during the PC to elucidate the human relevance of liver tumours observed in rodents are in the following Table 8.

Table 8: *mechanistic studies submitted during the PC*

Test substance **Route** **Duration of study** **Type of study**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Dose levels** **Issues addressed**	**Main findings**	**Reference**
thiophanate-methyl *In vitro* assay	Rodents (rat and mouse)	Activation of the aryl hydrocarbon receptor in liver tumour and its relevance to human. OECD guidance for AOP (Adverse Outcome Pathway) development helps to clarify the key biological event in the induction of tumour and its relevance for human.	AHR activation, weeks or months in duration, is necessary to induce rodent liver tumour promotion e hence, sustained AHR activation is deemed the molecular initiating event (MIE). Humans are less responsive than rodents and rodent species differ in sensitivity between strains.	Becker *et al.*, 2015

Results from a workshop on the human relevance of rodent liver tumors occurring via nongenotoxic modes of action	Rodents (rat and mouse)	Human relevance of rodent liver tumours involving the nuclear receptor-mediated MOAs of Constitutive Androstane Receptor (CAR) and Peroxisome Proliferator Activated Receptor–alpha (PPARα), and cytotoxicity.	CAR MOA is not qualitatively relevant to humans. Cytotoxicity is clearly relevant to humans, but a threshold applies.	Felter *et al*., 2018
Sodium phenobarbital (NaPB) at 0 (control), 200, 500, 750 and 1 000 ppm for 7 days in diet	WT (C57BL/6J) and hCAR/hPXR mice male C57BL/6J wild type (WT) mice and humanized mice, where both the mouse CAR and pregnane X receptor (PXR) have been replaced by their human counterparts (hCAR/hPXR mice) and cultured male C57BL/5J and CD-1 mouse, male Sprague-Dawley rat and male and female human hepatocytes.	Hepatic effects of sodium phenobarbital	In summary, although human hepatocytes are refractory to the mitogenic effects of NaPB, treatment with NaPB induced RDS *in vivo* in hCAR/hPXR mice, which is presumably due to the human CAR and PXR receptors operating in a mouse hepatocyte regulatory environment. As the response of the hCAR/hPXR mouse to the CAR activator NaPB differs markedly from that of human hepatocytes, the hCAR/hPXR mouse is thus not a suitable animal model for studies on	Haines. *et al*., 2018

			the hepatic effects of non-genotoxic rodent CAR activators.	
In vivo study by intra peritoneal injection at (80 mg/kg/ bw/d for 4 days of phenobarbital (concurrent vehicle: saline)	Homozygous humanized and knockout mice for CAR and PXR (huPXR/huCAR, PXRKO CARKO)	Relevance of mouse non genotoxic hepatocarcinogen induced by phenobarbital and chlordane induced hepatomegaly characterized by hypertrophy and hyperplasia in humans	We cannot be certain that hCAR and hPXR when expressed in the mouse can function exactly as the genes do when they are expressed in human cells. However, all parameters investigated to date suggest that much of their functionality is maintained.	Ross *et al*., 2010
Collection of multiplexed gene expression assays focused on Phase I and II xenobiotic metabolizing enzymes and transporters	Human primary cell cultures were treated with chemicals (309 environmental chemicals) at 5 concentrations (0.004-40 mM) for 6, 24 and 48 h.			Shah *et al*., 2011
Opinion of the Applicant on the carcinogenicity of thiophanate-methyl	Analysis of all data	No relevance for human of thyroid and liver tumours observed in rodents.	The only tumour type observed at higher frequency than in controls was thyroid follicular cell adenoma in rats and hepatocellular adenoma in mice.	Briese, *et al.,* 2018a

The studies reported above were submitted by Industry in order to support the hypothesis that phenobarbital and thiophanate-methyl induce tumours in rodents with similar modes of action, namely through the involvement of nuclear receptors CAR/PXR. However, these studies provide mostly information of a more general nature, without thiophanate-methyl specific data in support, so RAC agrees with the DS that the lack of human relevance is not demonstrated. Moreover, there is no demonstration that this is the only mechanism responsible for the induction of tumours in mice. Anyway, the hypothesis that adverse effects on the thyroid observed in rats are solely secondary to effects in the liver is not scientifically justified as in this case clear effects in the liver of rats would be expected. In addition, it should be noted that an investigation on the effects of antiepileptic drugs, including phenobarbital, showed an association with hypothyroidism in patients, indicating that also liver enzyme induction is of relevance in humans (Lai *et al.*, 2013).

Assessment and comparison with the classification criteria

RAC agrees with the argumentation of the DS about the relevance of the thyroid effect observed in rodents for humans (see RCOM). However also in the case that TPO is not involved in the observed adverse outcomes observed in rodents (i.e. hypertrophy and hyperplasia of thyroid), the relevance of these effects for humans cannot be excluded. These effects are considered relevant for human health, regardless of whether they are due to a direct action on the thyroid, or indirectly via liver induction or other mechanisms. In this regard, as suggested by the DS, the investigation of potential interference on the iodine uptake looking at the activity of the Na/I symporter deserve further attention to establish a mechanism of action potentially relevant across all species.

Regarding the possible involvement of nuclear receptors CAR, PPRAα, PXR and AhR in the etiology of tumours induced in mice by thiophanate-methyl, RAC agrees with the DS that in the absence of data on the simultaneous activation of the four NR the lack of human relevance is not demonstrated. Moreover, there is no demonstration that this is the only mechanism responsible for the induction of tumours in mice. Anyway, the hypothesis that adverse effects on the thyroid observed in rats are solely secondary to effects in the liver is not scientifically justified as in this case clear effects in the liver of rats would be expected.

As there is no human data available for thiophanate-methyl that may be relevant for carcinogenicity, the classification criteria for subcategory 1A are not fulfilled.

Regarding classification in category 1B or category 2, the significance of tumours observed in the chronic and carcinogenicity studies, i.e. thyroid follicular cell adenomas in rats and hepatocellular adenomas in mice is discussed below:

(a) tumour type and background incidence;

Rats: Induction of thyroid follicular cell adenomas was observed; this was significant only at the top dose, where the MTD was probably exceeded. At this dosage also three cases of adenocarcinoma were reported. The study report does not include historical control data.

Mice: Induction of hepatocellular adenomas was observed with statistically significant increases over the historical control at the two higher doses. The MTD was not reached.

(b) multi-site responses;

Rats: Statistically significant increases only of thyroid follicular cell adenoma in males were noted.

Mice: Statistically significant increases only of hepatocellular adenoma in both sexes were noted.

(c) progression of lesions to malignancy;

Rats: The observation of 3/60 follicular cell adenocarcinomas in the high dose males group compared to none in controls, although was not statistically significant was reported at a dosage probably exceeding the MTD andmay be indicative of progression to malignancy.

Mice: Hepatocarcinoma was observed in one male of the high dose group (7 000 ppm) and in one male of the second lowest (640 ppm) dose group. As a clear dose-response was not observed in mice, in this species the indication of progression into malignancy is weaker than in rats.

(d) reduced tumour latency;

Rats: Follicular cell hyperplasia and hypertrophy were noted in both sexes from the two highest dose groups both at interim sacrifice (12 months) and at terminal sacrifice (24 months). Follicular cell adenocarcinomas were only observed at terminal sacrifice indicating slow tumour development.

Mice: At interim sacrifice (9 months), an increase in hepatocellular centrilobular hypertrophy was observed in mice from the two highest treatment groups and mid-dose females. Moreover, a higher severity grade was observed in high dose animals and in the males of the next highest treatment group. Hepatocellular adenomas were only observed at terminal sacrifice indicating slow tumour development.

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

Rats: Thyroid follicular cell adenomas statistically significant only in males

Mice: Hepatocellular adenomas in both sexes

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

Follicular cell adenocarcinomas were observed only in rats; hepatocellular adenomas were observed only in mice.

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

As suggested by a MSCA, in an EPA report on the carcinogenicity of thiocarbonyl compounds the type of carcinogenicity observed after treatment of these compounds is similar as what observed for thiophanate-methyl: predominantly thyroid hypertrophy/hyperplasia associated with inhibition of thyroid hormones resulting in tumours, but also liver tumours in mice. This similarity suggests a possible common mechanism of carcinogenicity also considering that both thiourea and thiouracile are goitrogenic in humans.

(h) routes of exposure;

Information restricted to studies performed using oral administration (via diet).

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

A comparative *in vitro* metabolism study is available (RAR Vol 3, B.6.1.4) and showed no differences in metabolism between human and rat microsomes.

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

Rats: Follicular cell adenomas of the thyroid were only statistically significant in animals administered the highest dietary concentration (6 000 ppm). Adenocarcinomas were also observed only at this dose level. At this dose level, the MTD was probably exceeded (53 of 55 males died). Therefore, this study does not provide a conclusive evidence of a carcinogenic effect. However, overall the study shows an apparent progression of carcinogenic effects (hypertrophy-hyperplasia-adenoma-adenocarcinoma) in relation with dosage and timing, therefore these findings should not be disregarded.

Mice: Hepatocellular adenomas occurred in all treated animals, with statistically significant increases in the two highest treatment groups (3 000 and 7 000 ppm) at administration level that are considered below the MTD.

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

The substance induces aneuploidy in somatic cells and could thus be suspected to have a carcinogenic potential at levels above a threshold level. However, since only a single tumour type was observed in rats and mice there are no clear indications of a link between genetic instability and cancer.

Rats: Increased thyroid weight with hypertrophy/hyperplasia and sometimes accompanying effects on thyroid hormones were observed in many of the repeated dose toxicity studies available (including studies on short-term toxicity, long-term toxicity reproduction toxicity and neurotoxicity). In many of the studies there was also effects on the liver which could possibly indicate an up-regulation of the thyroid in response to increased hepatic clearance of T4 by uridine diphospho-glucuronosyltransferase (UDPGT). This is considered a rodent-specific effect generally of lack relevance for humans. The study also showed that phenobarbital, which was used as a reference substance, also induced liver cell proliferation and the drug metabolizing enzymes but caused only a very slight increase of TSH without thyroid hypertrophy, further suggesting that the increased UDPGT was not the only mechanism behind the thyroid effects. Anyway, the hypothesis that adverse effects on the thyroid observed in rats are solely secondary to effects in the liver is not scientifically justified as in this case clear effects in the liver of rats would be expected. The inhibition of hormone synthesis in the thyroid due to an inhibition of TPO has been considered the main cause of the T4 depression. However also in the case that thiophanate-methyl does not inhibit TPO activity in humans, as indicated by recent *in vitro* data on human cells provided during PC, other thiophanate-methyl dependent mechanisms could lead to unbalanced thyroid hormone levels and hence to the observed hypertrophy and hyperplasia of the gland. Moreover, as suggested by the DS in the

RCOM, the investigation of the iodine uptake by the Na/I symporter deserve further attention to establish a mechanism of action potentially relevant across all species. Therefore, the available data do not allow to exclude that the thyroid effects observed could be relevant for humans.

Mice: Data in the mechanistic study suggest that hepatocellular adenomas result from induction of the cytochrome P450 drug metabolising system. However, the data available to support this claim is limited to an increased liver weight and proliferation of liver cells in mice treated with thiophanate-methyl or phenobarbital. This is not considered to conclusively link tumours to a phenobarbital-like mode of action lacking human relevance.

Dog: Supporting studies in dog for carcinogenic effects were evaluated. A moderate hypertrophy of the thyroid was noted in males and females at 50 and 250 mg/kg bw/d based on organ weight and histopathological examination. Effects on T4, T3 and TSH were also seen in 1 year study in dogs (B.6.3.2.5 in the RAR and also reported in the STOT RE section of this document). A moderate hypertrophy of the thyroid was noted in males and females at 50 and 250 mg/kg bw/day based on organ weight and histopathological examination was observed in a Beagle dogs 24 months.

The study on rats demonstrated a statistically significant increase of thyroid tumours only at a dosage probably exceeding the maximum tolerated dose. The study on mice reported hepatocellular adenomas below the maximum tolerated dose. The hepatocellular carcinomas observed in mice were not clearly dose-dependent. It was noted that the mode of action is not clear. Based on the observed hepatocellular adenomas with the supporting information from the identified thyroid follicular cell changes in rats with statistical significance at the two top-level doses of 1 200 ppm and 6 000 ppm, RAC concluded that the **Thiophanate-methyl warrants classification as Carc. 2; H351 (suspected human carcinogen)**

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 43: Summary table of animal studies on adverse effects on sexual function and fertility

Test substance Test type Method Guideline	Route of exposure	Species Strain Sex No./group Vehicle	Exposure Period	Doses	Main findings	Reference
Thiophanate-methyl (Topsin M*) 2-generation reproduction study OECD 416 Acceptable	Oral (in diet)	Rat Sprague-Dawley Crl:CD (SD)BR M, F 25/sex/dose	P (M, F): 14 weeks prior to mating, during mating and until sacrifice after weaning of the Fl generation. F1 (M, F): 14 weeks maturation period after weaning, during mating and until weaning of the F2a generation. Because of an unusually high	0, 200, 630 and 2000 ppm (equivalent to 0, 14.6, 46.0 and 147.1 mg/kg bw/day in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/day in females)	<u>Parental:</u> <u>200 ppm:</u> ↑ thyroid weight (F: 18%) - histopathological changes in the thyroid (hypertrophy, hyperplasia) and liver (hypertrophy) <u>Notable effects at higher doses:</u> ↑ thyroid and liver weights at 630 and 2000 ppm	RAR Vol 3 B.6.6.1.1

			pup loss during the weaning period of the F2a pups a second mating was performed. The Fl animals were maintained on test substance/diet for six weeks and then allowed to mate with the same partner as during the first mating to produce an F2b-litter. Exposure was continued until sacrifice after weaning of the F2b generation.		- hepatocyte hyper-trophy and thyroid hypertrophy and hyperplasia at 630 and 2000 ppm ↑ TSH at 2000 ppm ↓ T_4 at 2000 ppm (M) ↓ bw gain at 2000 ppm <u>Offspring:</u> <u>200 ppm:</u> ↓ bw (-9%) <u>Notable effects at higher doses:</u> ↓ bw (-10-14%at 630 ppm; -13-16% at 2000 ppm)	
Thiophanate-methyl 3-generation reproduction study OECD 416 with deviations Supportive	Oral (in diet)	Rat CD 10M, 20F/dose	F0 animals received the test substance for 60 days prior to mating and during the mating period. Following weaning of the first litter (Fla), the F0 mated again, thus producing the Flb litter. From the Flb litter 10 males and 20 females were maintained on dietary treatment for 60 days before they were allowed to mate. After weaning of the F2a litter, the animals of the Flb litter remated, resulting in the F2b litter. With the F2b litter the above described procedure was repeated and thus a third generation (F3a, F3b) was formed.	0, 40, 160 and 640 ppm (equivalent to approximately 0, 2.7, 10.6 and 43.3 mg/kg bw/day in males; 0, 3.1, 12.2 and 48.5 mg/kg bw/day in females)	No adverse effects were observed in the study.	RAR Vol 3 B.6.6.1.2

* The test substance was referred to as “Topsin M”. According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95.93 % in the two batches used in the study.

Table 44: Summary table of human data on adverse effects on sexual function and fertility

No data.

Table 45: Summary table of other studies relevant for toxicity on sexual function and fertility

No data.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Two studies on reproduction toxicity are available in rats. The studies are thoroughly presented in Annex I (Volume 3 to the RAR).

The first study was performed in accordance with GLP and mainly followed OECD 416. There were no adverse effects observed on fertility parameters. At 2000 ppm, pup weights were reduced by up to 11% (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16% reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12% during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13%; f: -16%) with no corresponding effect on maternal body weight. It is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The finding are therefore not considered relevant for classification.

The second study was not performed according to GLP and had several deviations compared to OECD 416. The purity of the test substance was not stated and the study is considered to provide supportive information only. No adverse effects on reproduction or parental toxicity were observed in the study and NOAEL for both effects were therefore was 640 ppm (corresponding to approximately 43 mg/kg bw/day in males and 49 mg/kg bw/day in females).

10.10.3 Comparison with the CLP criteria

According to CLP Guidance Annex I: 3.7.2.4.3, "*Classification is not necessarily the outcome in the case … when there is only a small reduction in foetal/pup weight …".*

A slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. Taken together, the reduced pup weight is therefore not considered to justify classification for reproductive toxicity.

10.10.4 Adverse effects on development

Table 46: Summary table of animal studies on adverse effects on development

Test substance **Test type** **Method** **Guideline**	**Route of exposure**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Exposure Period**	**Doses**	**Main findings**	**Reference**
Thiophanate-methyl (Topsin-M*) Developmental toxicity study OECD 414 Acceptable	Oral (gavage)	Rat COBS CD 25 dams/dose 5% aqueous Arabic gum	GD 6-19	0, 100, 300 and 1000, mg/kg bw/day	Maternal toxicity: 1000 mg/kg bw/day: ↓ adjusted bw gain (-22%) Developmental toxicity: No adverse effects	RAR Vol 3 B.6.6.2.2.1
Thiophanate-methyl Developmental toxicity study	Oral (gavage)	Rabbit New Zealand white Range-finding study:	GD 6-28	Range-finding study: 0, 10, 30, 100, 150, 300 and 600	**Range-finding study:** ↓ bw and food consumption at 30 mg/kg bw/day and above ↑ mortality at 150 mg/kg bw/day and above (1 at 150,	RAR Vol 3 B.6.6.2.2.2

Test substance **Test type** **Method** **Guideline**	**Route of exposure**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Exposure Period**	**Doses**	**Main findings**	**Reference**
OECD 414 Supportive		4 dams/dose <u>Main study:</u> 15 dams/dose 1% (w/v) aqueous methylcellulose suspension		mg/kg <u>Main study:</u> 0, 2, 6 and 20 mg/kg bw/day	1 at 300, 2 at 600 mg/kg/bw/day) **Main study:** <u>Maternal toxicity:</u> <u>6 mg/kg bw/day:</u> ↓ bw GD 6-12 (-30 g compared to pre-dosing) <u>20 mg/kg bw/day:</u> ↓ bw GD 6-12 (-130 g compared to pre-dosing) <u>Developmental toxicity:</u> <u>6 and 20 mg/kg bw/day:</u> ↑ supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis	
Thiophanate-methyl Developmental toxicity study OECD 414 Acceptable	<u>Range-finding study:</u> Oral (gavage) and dietary <u>Main study:</u> Oral (gavage)	Rabbit New Zealand white <u>Range-finding study:</u> 6 dams/dose <u>Main study:</u> 15 dams/dose 1% (w/v) aqueous methylcellulose suspension	<u>Range-finding study:</u> GD 6-18 <u>Main study:</u> GD 6-28	<u>Range-finding study:</u> 0, 5, 10, 20, 40 and 80 mg/kg/day <u>Main study:</u> 0, 5, 10, 20 and 40 mg/kg/day	**Range-finding study:** <u>Maternal toxicity:</u> <u>40 mg/kg bw/day:</u> ↑ abortions (3/6 does) (gavage group) ↓ bw and food consumption <u>80 mg/kg bw/day:</u> ↑ abortions (3/6 does) (gavage group) ↓ bw and food consumption <u>Developmental toxicity:</u> <u>80 mg/kg bw/day:</u> ↑ resorptions (gavage group) ↑ number of thoracic vertebrae ↓ number of lumbar vertebrae ↑ number of rib pairs **Main study:** <u>Maternal toxicity:</u> <u>20 mg/kg bw/day:</u> ↓ bw gain (-12%) compared to controls ↓ food consumption <u>40 mg/kg bw/day:</u> ↓ bw gain (-76%) compared to controls ↓ food consumption ↓ faecal output <u>Developmental toxicity:</u> <u>40 mg/kg bw/day:</u> ↑ supernumerary thoracic ribs	RAR Vol 3 B.6.6.2.2.3
Thiophanate-	Oral	Mouse	GD 1-15	0, 40, 200,	<u>Maternal toxicity:</u>	RAR Vol 3

Test substance **Test type** **Method** **Guideline**	**Route of exposure**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Exposure Period**	**Doses**	**Main findings**	**Reference**
methyl Developmental toxicity study OECD 414 with deviations Supportive	(gavage)	ICR 20 females/dose 5% aqueous Arabic gum suspension		500 and 1000 mg/kg bw/day	No adverse effects observed <u>Developmental toxicity:</u> <u>1000 mg/kg bw/day:</u> ↓ number of living foetuses	B.6.6.2.2.5

* The test substance was referred to as "Topsin M". According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 97.2%.

Table 47: Summary table of human data on adverse effects on development

No data.

Table 48: Summary table of other studies relevant for developmental toxicity

No data.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Studies are available on developmental toxicity in rats, rabbits and mice with the rabbit being the most sensitive species. The studies are thoroughly presented in Annex I (Volume 3 to the RAR).

In the rat study, performed in accordance with GLP and OECD 414, no adverse effects on development were observed at doses up to 1000 mg/kg bw/day. At this dose, there was a 22% reduction in adjusted body weight gain. NOAEL for maternal toxicity was thus found to be 300 mg/kg bw/day.

In the first rabbit study, also performed in accordance with GLP and OECD 414, there was a positive trend for supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis. NOAEL for developmental toxicity was 2 mg/kg bw/day based on these findings. NOAEL for maternal toxicity was 2 mg/kg bw/day based on slight reductions in body weight at 6 mg/kg bw/day. The study is considered to be supportive as it seems some animals may have suffered from infections (please refer to study summary in Annex I).

The second rabbit study was performed in accordance with GLP and OECD 414 and gave higher NOAEL values than the first rabbit study. NOAEL for developmental toxicity was 20 mg/kg bw/day based on supernumerary thoracic ribs at 40 mg/kg bw/day and NOAEL for maternal toxicity was 10 mg/kg bw/day based on minimal, transient but significant reduction in maternal body weight gain and significantly reduced absolute and relative feed consumption values for the entire dosage period at 20 mg/kg bw/day. The 40 mg/kg/day dosage produced adverse clinical observations (reduced faecal output) and significantly reduced body weight gain and absolute and relative feed consumption values for the entire dosage period.

The applicant[4] has submitted a statement discussing the different results obtained in the two rabbit studies. The overall summary and conclusion provided by the applicant was the following:

> *"Both studies were critically reviewed to examine the relative strength of each study and to identify potential causes of observations unrelated to the test substance (e.g., intercurrent disease;*

[4] The applicant in the EFSA process, *i.e.* applying for renewal of approval as an active substance in plant protection products.

spontaneous differences in litter sizes; historical experience and techniques of the Testing Facility). Based on my critical review of both studies, it is my opinion that the appropriate maternal NOAEL for thiophanate-methyl in pregnant rabbits is 10 mg/kg/day, and the appropriate developmental NOAEL is greater than 20 mg/kg/day. Transient, significant maternal weight loss, an apparent effect at 20 mg/kg/day in the first study, did not occur at this dosage in the second study, even though a prolonged dosage period was used, indicating that compromised maternal health was a probable confounder in the first study. A dosage of 40 mg/kg/day was required to produce transient significant maternal weight losses followed by a significant reduction in maternal body weight gain in the second study. Transient, significant reductions in feed consumption values were produced by 20 and 40 mg/kg/day dosages in the second study, and the 40 mg/kg/day dosage also reduced faecal output.

Reanalysis of the foetal data from the first study indicates that the foetal observations were probably associated with deficient maternal health, technical problems, outlier observations and/or the relatively few animals available for evaluation. No foetal alterations attributable to thiophanate-methyl dosages as high as 20 mg/kg/day occurred in the second study. The results of the second study identify the developmental NOAEL as greater than 20 but less than 40 mg/kg/day. Developmental toxicity at the 40 mg/kg/day dosage consisted of increased incidences of extra thoracic ribs (supernumerary ribs), a reversible variation in ossification. Although this variation was identified as an effect of 6 and 20 mg/kg/day dosages of thiophanate-methyl in the first study, reanalyses of the data identifies that the incidences were within expected normal ranges and not statistically significant. All other variations in skeletal ossification identified in the first study also occurred at incidences that were not statistically significant and were not replicated in the second study, indicating that they were unrelated to the test substance.

It is my conclusion that appropriate maternal and developmental NOAELs for the rabbit should be based on the results of the more robust second study, in which larger numbers of maternal rabbits and foetuses were evaluated, a longer treatment period was used, and regarding foetal evaluations, analyses were based on the more appropriate unit, the litter."

The dossier submitter agrees that the second study (RAR Vol 3 B.6.6.2.2.3) was of higher reliability and that effects were seen at higher doses. It is unclear how the seemingly compromised maternal health in the first study (RAR Vol 3 B.6.6.2.2.2) affected the outcome of the study. The applicant was asked to recalculate the effects seen in thefirst study to obtain the grand litter mean. Statistical analyses showed a statistically significant dose-related trend supernumerary ribs, thickened ribs, 27 presacral vertebrae and asymmetric pelvis. The same effect, supernumerary ribs with an associated increase in the average for thoracic vertebrae, were noted in both studies. The first study is therefore considered to support this finding in the more robust second study.

The mouse study is considered to provide supportive information only as it was not performed in accordance with GLP and did not comply with OECD 414. No maternal toxicity was observed at doses up to 1000 mg/kg bw/day. At this dose, the average number of living foetuses was slightly but significantly ($p<0.05$) lower (9.7%), compared to the control group (10.9%). NOAEL for developmental toxicity is therefore considered to be 300 mg/kg bw/day.

10.10.6 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on eveidence from humans or in 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) largely based on animal data. The animal data required for iB classification "*shall provide clear evidence of an adverse effect on sexual function or on development in the absence of other toxic effects, or if occurring together wiht other toxic effects the averse effect on reproduction is considered not to be a secondary non-specific consequence of tother toxic effects*".

Substances are classified in Category 2 when there is *"some evidence from humans or experimental animals… of an adverse effect on sexual function or on development, and where the evidence is not sufficiently convincing to place the substancve in Category 1."*

As there is no human data available for thiophanate-methyl, the criteria for category 1A are not fulfilled. The effects noted in the studies that are considered potentially relevant for classification are abortions and skeletal effects.

Abortions

Abortions were noted in both developmental toxicity studies in rabbits. In the first rabbit study, a high level of foetal loss was observed in the range-finding study at 100 mg/kg bw/day and above. This dose level is close to the dose where mortality occurred (starting from 150 mg/kg bw/day) and this effect is therefore not considered relevant for the purpose of classification. One litter was lost by abortion on GD 23 at 30 mg/kg bw/day, also in the range-finding study. This was within the background control range, but involvement with treatment could not be excluded in view of the results obtained at the higher doses. This doe had a body weight on GD 20 that was reduced by 21% compared to the control group. In the main part of the study, one control, one low-dose, one mid-dose and two high-dose animals aborted, although the reasons for these findings remain unclear due to the possibly compromised maternal health status.

Abortions were also seen in the second developmental toxicity study. 3/6 does aborted at both 40 and 80 mg/kg bw/day in the range-finding study. Body weights, body weight gains and feed consumption values for the does that aborted were comparable to other does in the same dosage groups and there were no clinical observations prior to aborting. In the 40 mg/kg bw/day dose group there was a slight reduction in body weight gain compared to controls that was not considered adverse. At 80 mg/kg bw/day there was an adverse reduction in body weight gain compared to controls (-19%). The abortions in the range-finding study were considered treatment-related. However no abortions were observed at 40 mg/kg bw/day (the highest dose) in the main part of the study. It could possibly be discussed whether these findings would support a classification in Repr. 2 but the dossier submitter does not consider it justified as the abortions were not observed in the main part of the most reliable study.

Skeletal effects

Three studies on the developmental toxicity of thiophanate-methyl are available, one in rats and two in rabbits. No signs of developmental toxicity was observed in the rat study. In both rabbit studies there was an increased incidence in supernumerary ribs with an associated increase in thoracic vertebrae. In the first rabbit study, there was also a statistically significant trend observed for thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis. These effects appeared to be dose-related and started at a dose with no apparent maternal toxicity; maternal body weight was reduced by 30 g on GD 6 at 6 mg/kg bw/day where the skeletal effects started and by approximately 180 g on GD 10 at the higher dose of 20 mg/kg bw/day, body weights then recovered and reached levels comparable to the control. The reliability of this study was questioned due to indications of impaired maternal health as several of the animals appeared to have suffered from infections.

In the second study, supernumerary ribs with an associated increase in thoracic vertebrae was also observed but occurred at a dose level which also produced maternal toxicity shown as reduced body weight gain (-76% compared to controls, $p<0.01$). It therefore seems likely that the effect was secondary to maternal toxicity.

According to CLP Guidance Annex I: 3.7.2.4.3, "*Classification is not necessarily the outcome in the case of minor developomental changes, when there is only a small reduction in foetal/pup weight or retardation of ossification seen in association with maternal toxicity".*

Conclusion on classification for developmental toxicity

In conclusion, the effects observed in the studies with thiophanate-methyl are not considered to be neither "clear" nor "some" evidence of an adverse effect on development and the criteria for classification are thus not considered to be met.

10.10.7 Adverse effects on or via lactation

Table 49: Summary table of animal studies on effects on or via lactation

Test substance **Test type** **Method** **Guideline**	**Route of exposure**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Exposure Period**	**Doses**	**Main findings**	**Reference**
Thiophanate-methyl (Topsin M*) 2-generation reproduction study OECD 416 Acceptable	Oral (in diet)	Rat Sprague-Dawley Crl:CD (SD)BR M, F 25/sex/dose	P (M, F): 14 weeks prior to mating, during mating and until sacrifice after weaning of the Fl generation. F1 (M, F): 14 weeks maturation period after weaning, during mating and until weaning of the F2a generation. Because of an unusually high pup loss during the weaning period of the F2a pups a second mating was performed. The Fl animals were maintained on test substance/diet for six weeks and then allowed to mate with the same partner as during the first mating to produce an F2b-litter. Exposure was continued until sacrifice after weaning of the F2b generation.	0, 200, 630 and 2000 ppm (equivalent to 0, 14.6, 46.0 and 147.1 mg/kg bw/day in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/day in females)	Parental: 200 ppm: ↑ thyroid weight (F: 18%) - histopathological changes in the thyroid (hypertrophy, hyperplasia) and liver (hypertrophy) Notable effects at higher doses: ↑ thyroid and liver weights at 630 and 2000 ppm - hepatocyte hyper-trophy and thyroid hypertrophy and hyperplasia at 630 and 2000 ppm ↑ TSH at 2000 ppm ↓ T_4 at 2000 ppm (M) ↓ bw gain at 2000 ppm Offspring: 200 ppm: ↓ bw (-9%) Notable effects at higher doses: ↓ bw (-10-14%at 630 ppm; -13-16% at 2000 ppm)	RAR Vol 3 B.6.6.1.1
Thiophanate-methyl 3-generation reproduction study OECD 416 with deviations	Oral (in diet)	Rat CD 10M, 20F/dose	F0 animals received the test substance for 60 days prior to mating and during the mating period. Following weaning of the first litter (Fla), the F0 mated	0, 40, 160 and 640 ppm (equivalent to approximately 0, 2.7, 10.6 and 43.3 mg/kg bw/day in males; 0, 3.1, 12.2 and 48.5 mg/kg bw/day in females)	No adverse effects were observed in the study.	RAR Vol 3 B.6.6.1.1

Supportive			again, thus producing the Flb litter. From the Flb litter 10 males and 20 females were maintained on dietary treatment for 60 days before they were allowed to mate. After weaning of the F2a litter, the animals of the Flb litter remated, resulting in the F2b litter. With the F2b litter the above described procedure was repeated and thus a third generation (F3a, F3b) was formed.			

* The test substance was referred to as "Topsin M". According to the applicant, this is a synonym for thiophanate-methyl. The purity of thiophanate-methyl was 95.93 % in the two batches used in the study.

Table 50: Summary table of human data on effects on or via lactation

No data.

Table 51: Summary table of other studies relevant for effects on or via lactation

No data.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Reduced pup weight was noted in the 2-generation study in rats (RAR Vol 3 B.6.6.1.1). At 2000 ppm, pup weights were reduced by up to 11% (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16% reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12% during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13%; f: -16%) with no corresponding effect on maternal body weight.

10.10.9 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP) Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

"(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criterias (a) and (c).

A slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The reduced body weight gain is therefore not considered "provide clear evidence of adverse effect in the offspring due to transfer in the milk".

10.10.10 Conclusion on classification and labelling for reproductive toxicity

No classification is proposed for thiophanate-methyl.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Reproductive toxicity

Adverse effects on sexual function and fertility

Two studies on fertility are available in rats.

A 2-generation study was performed in rats Sprague-Dawley Crl:CD (SD)BR oral (in diet) at 0, 200, 630 and 2 000 ppm (equivalent to 0, 14.6, 46.0 and 147.1 mg/kg bw/d in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/d in females) in accordance with GLP and OECD TG 416 at the time (TG of 1983). In this study, there were no adverse effects observed on fertility parameters. At 2 000 ppm (the highest dose tested), pup weights were reduced by up to 11 % (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16 % reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12 % during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13 %; f: -16 %) with no corresponding effect on maternal body weight. It is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The finding are therefore not considered relevant for classification.

A 3-generation study was not performed according to GLP and had several deviations compared to OECD TG 416 and it is considered only supportive. The purity of the test substance was not stated. No adverse effects on reproduction or parental toxicity were observed in the study and NOAEL for both effects therefore was the top dose of 640 ppm (corresponding to approximately 43 mg/kg bw/d in males and 49 mg/kg bw/d in females).

Conclusion

In the absence of adverse effects on fertility parameters, the DS proposed no classification for effects on sexual function and fertility.

Developmental toxicity

Studies are available on developmental toxicity in rats, rabbits and mice with the rabbit being the most sensitive species. (RAR, Volume 3).

In the rat study (RAR Vol. 3 B.6.6.2.2.1), performed in accordance with GLP and OECD

TG 414, no adverse effects on development were observed at doses up to 1 000 mg/kg bw/d. At this dose, there was a 22 % reduction in adjusted body weight gain. NOAEL for maternal toxicity was thus found to be 300 mg/kg bw/d.

In the first rabbit study (RAR Vol. 3 B.6.6.2.2.2), the main study also performed in accordance with GLP and OECD TG 414, there was a positive trend for supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis. NOAEL for developmental toxicity was 2 mg/kg bw/d based on these findings. NOAEL for maternal toxicity was 2 mg/kg bw/d based on slight reductions in body weight at 6 mg/kg bw/d. The study is considered to be supportive as it seems some animals may have suffered from infections.

The second rabbit study (RAR Vol. 3 B.6.6.2.2.3) was performed in accordance with GLP and OECD TG 414 and gave higher NOAEL values than the first rabbit study. NOAEL for developmental toxicity was 20 mg/kg bw/d based on supernumerary thoracic ribs at 40 mg/kg bw/d and NOAEL for maternal toxicity was 10 mg/kg bw/d based on minimal, transient but significant reduction in maternal body weight gain and significantly reduced absolute and relative feed consumption values for the entire dosage period at 20 mg/kg bw/d. The 40 mg/kg bw/d dosage produced adverse clinical observations (reduced faecal output) and significantly reduced body weight gain and absolute and relative feed consumption values for the entire dosage period.

Both rabbit studies were critically reviewed to examine the relative strength of each study and to identify potential causes of observations unrelated to the test substance (e.g., inter-current disease; spontaneous differences in litter sizes; historical experience and techniques of the testing facility). In the CLH report, the DS reported a critical review of the Applicant that was of the opinion that the appropriate maternal NOAEL for thiophanate-methyl in pregnant rabbits is 10 mg/kg bw/d, and the appropriate developmental NOAEL is greater than 20 mg/kg bw/d. Transient, significant maternal weight loss, an apparent effect at 20 mg/kg bw/d in the first study, did not occur at this dosage in the second study, even though a prolonged dosage period was used, indicating that compromised maternal health was a probable confounder in the first study. A dosage of 40 mg/kg bw/d was required to produce transient significant maternal weight losses followed by a significant reduction in maternal body weight gain in the second study. Transient, significant reductions in feed consumption values were produced by 20 and 40 mg/kg bw/d dosages in the second study, and the 40 mg/kg bw/d dosage also reduced faecal output.

Re-analysis of the foetal data from the first study indicated that the foetal observations were probably associated with deficient maternal health, technical problems, outlier observations and/or the relatively few animals available for evaluation. No foetal alterations attributable to thiophanate-methyl dosages as high as 20 mg/kg bw/d occurred in the second study. The results of the second study identified the developmental NOAEL as greater than 20 but less than 40 mg/kg bw/d. Developmental toxicity at the 40 mg/kg bw/d dosage consisted of increased incidences of extra thoracic ribs (supernumerary ribs), a reversible variation in ossification. Although this variation was identified as an effect of 6 and 20 mg/kg bw/d doses of thiophanate-methyl in the first study, re-analyses of the data identified that the incidences were within the expected normal ranges and not statistically significant. All other variations in skeletal ossification identified in the first study also occurred at incidences that were not statistically

significant and were not replicated in the second study, indicating that they were unrelated to the test substance. Abortions were seen in the dose range finding studies at toxic doses but not in the main studies, thus not considered relevant for classification.

The mouse study is considered to provide supportive information only as it was not performed in accordance with GLP and did not comply with OECD TG 414. No maternal toxicity was observed at doses up to 1 000 mg/kg bw/d. At this dose, the average number of living foetuses was slightly but significantly ($p < 0.05$) lower (9.7), compared to the control group (10.9). NOAEL for developmental toxicity was therefore considered to be 300 mg/kg bw/d.

Conclusion

The effects noted in the studies that are considered potentially relevant for classification are abortions in dose range finding studies but not in the main studies and skeletal variations. Regarding the abortions the DS did not consider the findings sufficient to justify a classification as Repr. 2 for developmental effects. The observed skeletal effect were considered by the DS secondary to maternal toxicity and therefore not sufficient to trigger classification.

Lactation

Reduced pup weight was noted in the 2-generation study in rats. At 2 000 ppm, pup weights were reduced by up to 11 % (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16% reduction and statistically significant in F2b) but also reduced maternal body weight gain F1 females (-12 % during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13 %; f: -16 %) with no corresponding effect on maternal body weight.

Conclusion

According to the CLP Regulation Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

> *"(a) human evidence indicating a hazard to babies during the lactation period; and/or*
> *(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*
> *(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."*

No data are available to address criteria (a) and (c).

A slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The reduced body weight gain is therefore not considered "provide clear evidence of adverse effect in the offspring due to transfer in the milk".

Comments received during public consultation

Three comments were received on reproductive toxicity during public consultation. Two MSCAs agreed with no classification, while one MSCA stated that, being the carbendazim a major metabolite of thiophanate-methyl and as this substance is classified as Repr. 1B, information available for carbendazim should be considered as relevant and discussed here.
The DS considered that, as the studies on reproductive toxicity with thiophanate-methyl in rats and rabbits available are negative, the data on carbendazim should only be considered if differences in metabolism between rats/rabbits and humans are expected, with higher levels of carbendazim formed in humans. There were no such indications in the comparative *in vitro* metabolism study. The studies on thiophanate-methyl itself are therefore considered to be the most relevant for classification of thiophanate-methyl.

Moreover, the following was concluded at the EFSA Expert meeting for thiophanate-methyl: "Although it is acknowledged that thiophanate-methyl produces the metabolite carbendazim that is classified as Repr. Cat 1B, and therefore a concern cannot be completely excluded, the majority of the experts agreed that the data base is sufficient for excluding classification of the substance regarding toxicity for reproduction."

Assessment and comparison with the classification criteria

Fertility

Both generation studies were performed at low doses (no toxicity observed at any doses). It should be noted that OECD TG 416 recommends that the highest dose level should be chosen with the aim to induce toxicity, while in both studies the dosages did not reach toxic levels. Furthermore, the fact that carbendazim, a metabolite of thiophanate-methyl, adversely affects spermatogenesis, raises a concern about lack of investigation of sperm parameters in the generational studies with thiophanate-methyl.

In conclusion, the available experimental studies are considered by RAC to be inconclusive due to inadequate dosing and therefore no classification due to lack of data is the only conclusion possible for fertility.

Developmental toxicity

In the 2-generation study, a slight reduction in pup weight at 14-21 days was observed that could not be entirely explained by maternal toxicity. However, it is possible that the pups consumed the thiophanate-methyl containing feed and thus were exposed directly through the feed. The reduced pup weight is therefore not considered by RAC to justify classification for developmental toxicity.

Developmental toxicity studies are available in rats, mouse and rabbits. The most sensitive species is the rabbit were abortion and skeletal variations were recorded in the presence of maternal toxicity. The skeletal effects in study B.6.6.2.3 are reported in the following tables:

Table B.6.2.2.3-1: Skeletal findings in dose-range finding study (stomach tube exposure)

Observation	Dose level (mg/kg bw/day)			Historical control range
Ossification sites per foetus per litter (mean ± SD)	**0**	**5**	**80**	
Number of ribs (pairs)	12.45 (±0.31)	12.24 (±0.15)	12.87 (±0.12)	12.34-12.67
Thoracic vertebrae	12.48 (±0.34)	12.34 (±0.22)	12.90 (±0.10)	12.38-12.70
Lumbar vertebrae	6.48 (±0.32)	6.66 (±0.22)	6.10 (±0.10)	6.30-6.61

Table B.6.2.2.3-2: Skeletal findings in main study

Observations	Dose level (mg/kg bw/day)				
Ossification sites per foetus per litter (mean ± SD)	**0**	**5**	**10**	**20**	**40**
Number of ribs (pairs)	12.45 (±0.28)	12.44 (±0.25)	12.45 (±0.25)	12.58 (±0.24)	12.85 (±0.15)**
Thoracic vertebrae	12.50 (±0.28)	12.52 (±0.30)	12.53 (±0.25)	12.68 (±0.22)	12.89 (±0.12)**
Lumbar vertebrae	6.48 (±0.28)	6.47 (±0.28)	6.46 (±0.24)	6.32 (±0.22)	6.09 (±0.12)**

** Statistically significantly different from the vehicle control group ($p<0.01$)

The abortions were considered not sufficient for classification as this effect was only seen at high toxicity in the preliminary studies, but not in the main studies. The skeletal effects also do not warrant classification, as they may be secondary to maternal toxicity and are considered as variations.

In conclusion, RAC is of the opinion that the available data are conclusive and that classification of thiophanate-methyl for developmental toxicity is not warranted.

Lactation

RAC agrees with the DS that classification for lactation is not warranted.

10.11 Specific target organ toxicity-single exposure

Table 52: Summary table of animal studies on STOT SE

Test substance/ Route	Vehicle	Method Guideline	Species Strain Sex No./dose	Dose levels	LD_{50}/LC_{50}	Reference
Thiophanate-methyl Oral	Distilled water	EPA OPP 81-1 (similar to OECD 401)	Rat Crj:CD(SD) M, F 5/sex	5000 mg/kg bw	>5000 mg/kg bw	RAR Vol 3 B.6.2.1
Thiophanate-methyl Dermal	Distilled water	EPA OPP 81-2 (similar to OECD 402)	Rabbit Kbs:JW 5/sex	2000 mg/kg bw	>2000 mg/kg bw	RAR Vol 3 B.6.2.2
Thiophanate-methyl Inhalation (4 hr, whole body, aerosol)	-	EPA OPP 81-3 (similar to OECD 403)	Rat Crj:CD(SD) M, F 5/sex	0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L	LC_{50} M: 1.7 mg/L LC_{50} F: 1.9 mg/L	RAR Vol 3 B.6.2.3

M: male; F: female

Test substance **Route** **Duration of study**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Dose levels**	**Main findings**	**Reference**
Thiophanate-methyl Acute neurotoxicity OECD 424 Oral (gavage)	Rat Sprague-Dawely M, F 10/sex/dose 0.5% Methylcellulose	0, 500, 1000 and 2000 mg/kg bw (main study) 0, 50, 125, 500 and 2000 mg/kg bw (extension study)	No adverse effects	RAR Vol 3 B.6.7.1.1
Thiophanate-methyl Oral (capsule) 83-1. The study fulfils OECD 409. 1 year	Dog Beagle M, F 4/sex/dose	0, 8, 40 and 200 mg/kg bw/day	<u>8 mg/kg bw/day:</u> - ↑ Thyroid weight in females (29%) <u>Notable effects at higher doses:</u> -Tremors shortly after dosing at 200 mg/kg bw/day - ↓ Bw at 200 mg/kg bw/day (-20%) - Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in males at 200 mg/kg bw/day - ↓ T_4 in males at 200 mg/kg bw/day - Histopathological changes in the thyroid (hypertrophy and hyperplasia)	RAR Vol 3 B.6.3.2.5
Thiophanate-methyl Oral (capsule) 90-day Acceptable	Dog Beagle M, F 4/sex/dose	0, 50, 200 and 400 mg/kg bw/day (800 mg/kg bw/day for the first 7 weeks)	<u>50 mg/kg bw/day:</u> - histopathological changes (minimal follicular cell hypertrophy) in the thyroid (1m, 1f) <u>Notable effects at higher doses:</u> - One high dose male sacrificed in moribund condition at 800 mg/kg bw/day - ↓ Bw at 200 (-15-24%) and 400 mg/kg bw/day (-26-28%) - ↓ food consumption at 200 and 400 mg/kg bw/day - Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in females at 200 and 400 mg/kg bw/day and males at 400 mg/kg bw/day - Changes in biochemical parameters consistent with malnutrition and dehydration - ↓ T_3 and T_4 in females at 200 and 400 mg/kg bw/day and ↓ T_3 in males at 400 mg/kg bw/day - ↑ Liver and thyroid weights - Histopathological changes in the thyroid. Hypertrophy: incidence (0/8, 2/8, 5/8, 8/8) and severity increased with increased dose. Hyperplasia in 0/8, 0/8, 1/8, 7/8 at the respective dose levels.	RAR Vol 3 B.6.3.2.4

Table 53: Summary table of human data on STOT SE

No data.

Table 54: Summary table of other studies relevant for STOT SE

No data.

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

In the acute oral a toxicity study no clinical signs were noted. In the dermal toxicity study, the only clinical sign that was noted was erythema which as a local irritancy effect is of no relevance for STOT SE classification (see section 10.1-2). In the acute inhalation toxicity study, several clinical signs were observed and are presented in the table below.

Table 55: Mortalities and clinical signs observed in acute inhalation toxicity study with thiophanate-methyl

Dose (mg/L)	Males		Females	
	Clinical signs	**Mortality**	**Clinical signs**	**Mortality**
0.5	Not tested		-	0/5
1.0	-	0/5	Decreased motor activity (2/5, 1 d) Convulsion (2/5, 1 d) Low sensitivity (2/5, 1 d)	1/5
1.5	Tremor (5/5, day 1)	0/5	Tremor (4/5, 1 d)	0/5
1.6	Decreased motor activity (5/5, 1-3 h) Tremor (5/5, 1-3 h) Low sensitivity (5/5, 1-3 h) Ptosis (5/5, 1 h)	0/5	Decreased motor activity (5/5, up to 2 d) Ventral position (2/5, 3 h – 1 d) Convulsion (2/5, 1-2 d) Tremor (5/5, 1 h - 1 d) Low sensitivity (5/5, up to 2 d) Incontinence of urine (2/5, 1 d) Ptosis (2/5, 1-3 h)	0/5
1.9	Decreased motor activity (2/4, 1-3 h) Convulsion (1/4, 1-3 h) Low sensitivity (2/4, 1-3 h) Ptosis (1/4, 3 h)	5/5	Decreased motor activity (3/5, 3 h - 1 d) Hypotonia (1/5, 3 h) Ventral position (1/5, 3 h) Convulsion (3/5, 3 h - 1 d) Ataxia (1/5, 3 h) Low sensitivity (3/5, 3 h - 1 d)	3/5

Two subchronic toxicity studies with thiophanate-methyl are available with dogs (see section 10.12). The only effect observed that could be of relevance to classification in STOT SE is tremors seen in the 1-year study. The same effect was however not observed in the 90-day study.

The 1-year study was performed in accordance with GLP and OECD 409 although following the EPA 83-1 guideline. Thiophanate-methyl was administered to Beagle dogs (Marshall research animals, Inc., North Rose New York). Four animals per sex and dose group received the test substance orally via gelatine capsules daily at dose levels of 0, 8, 40 and 200 mg/kg bw/day. All animals survived throughout the study. Tremors were seen in all high dose animals and one mid-dose animal shortly after dosing on one or more

occasions during the initial three weeks of study but were not observed subsequently. The tremors were noted in all high dose animals and one mid-dose animal which indicates a dose-response relationship.

The 90-day study was performed in accordance with GLP and OECD 409 although following the EPA 82-1 guideline. Beagle dogs (Marshall research animals, Inc., North Rose New York) received thiophanate-methyl daily for three months via gelatine capsules at dose levels of 0, 50, 200 and 800 mg/kg bw/day. At study week eight, the high dose (800 mg/kg bw/day) was reduced to 400 mg/kg bw/day and maintained at this dose level until the end of the administration period. Four animals per sex and dose group were used. No tremors were noted in this study.

Table 56: Incidence of tremors noted in 1 year dog study with thiophanate-methyl

Dose (mg/kg bw/day)	Animal no	Sex	Days of study on which observed		
			Slight	Moderate or no degree indicated	Severe/tonic convulsions
40	3604	F	13		
200	4101	M	7 13	1	
200	4102	M		1	
200	4103	M	7 13	1 4 6	
200	4104	M	7 12 13	1	
200	4601	F	7	1	
200	4602	F		1	
200	4603	F	2 13 16 17	1	2 16 17
200	4604	F		4	

It is unclear why tremors were seen in the 1-year study but not in the 90-day study in the same species and at the same dose level. The dossier submitter does not consider it a clear indication of a neurotoxic effect as there are no indications of neurotoxicity in other studies and there were no other effects noted in this study that would support such a conclusion. However, it is also not a clearly non-specific effect caused by general toxicity (animals were relatively unaffected by dose at the beginning of the study) or stomach pain (the substance is not corrosive or irritating). But if caused by the administration itself, the tremors should have been observed in all dose groups including controls. In conclusion, the tremors seen are considered to be toxicologically relevant but the significance of the finding is unclear.

10.11.2 Comparison with the CLP criteria

According to the Regulation (EC) No 1272/2008 (CLP), specific target organ toxicity (single exposure) categories 1 and 2 is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture, which are not covered by the other hazard classes. Category 3 covers transient effects, occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE).

Categories 1 and 2

In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. After inhalation exposure, clinical signs such as tremors, convulsions, low sensitivity, decreased motor activity, ptosis, ataxia and ventral position were observed at doses close to the LC_{50} value. According to the CLP Guidance, care should be taken not to give a "double classification" for the same effect and as these effects occurred close to the lethal doses they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE.

No adverse effects were noted in the acute neurotoxicity study. In the one-year dog study, tremors were seen shortly after dosing in a dose-response related manner during the initial three weeks of study but were not observed subsequently. No tremors were observed in a 90-day study in the same species and at the same dose level. The significance of the finding is unclear and in the dossier submitter's opinion does not justify classification in STOT SE. There were no relevant early effects noted in the other repeated dose studies.

According to the results of the available studies, thiophanate-methyl thereby does not fulfil the CLP criteria for STOT SE category 1 or 2.

Category 3

This evaluation is usually based primarlily on human data. No human data is available for thiophanate-methyl. However, appropriate animal data, e.g. clinical signs or histopathology data from acute inhalation studies can also be used if available.

According to the CLP Guidance section 3.8.2.3, clinical signs (e.g. dyspnoea, rhinitis etc) and histopathology (hyperemia, edema, minimal inflammation, thickened mucous layer) observed in inhalation toxicity studies may justify classification for RTI and lethargy, lack of coordination, loss of righting reflex and ataxia observed in animal studies may justify classficiation for NE. Such effects were not seen in the studies available with thiophanate-methyl and classification in this category is therefore not justified.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification is proposed for thiophanate-methyl.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

In the CLH report three animal studies were reported to evaluate the specific target organ toxicity after single exposure (see previous section). No effects warranting classification as STOT SE were seen in these studies.

Other non-acute studies were considered by DS for the evaluation of STOT SE.

Two sub-chronic toxicity studies with thiophanate-methyl are available with dogs (section 10.12 of CLH dossier). The only effect observed that could be of relevance to classification in STOT SE is tremors seen in the 1-year study. It is to be noted that the same effect was however not observed in the 90-day study.

The 1-year study was performed with Beagle dogs in accordance with GLP and following EPA OPP 83-1 guideline (similar to OECD TG 409). Thiophanate-methyl was administered to four animals per sex and dose group that received the test substance orally via gelatine capsules daily at dose levels of 0, 8, 40 and 200 mg/kg bw/d. All animals survived throughout the study. Tremors were seen in all high dose animals and one mid-dose animal shortly after dosing on one or more occasions during the initial three weeks of the study but were not observed subsequently. The tremors were noted in all high dose animals and one mid-dose animal which indicates a dose-response relationship.

In a 90-day study (in RAR section B.6.3.2.4) performed in accordance with GLP and following EPA OPP 83-1 guideline (similar to OECD TG 409). Beagle dogs received thiophanate-methyl daily for three months via gelatine capsules at dose levels of 0, 50, 200 and 800 mg/kg bw/d. At study week eight, the high dose (800 mg/kg bw/d) was reduced to 400 mg/kg bw/d and maintained at this dose level until the end of the administration period. Four animals per sex and dose group were used. No tremors were noted in this study. The incidence of tremors noted in 1-year dog study with thiophanate-

methyl is reported in the following table:

Dose (mg/kg bw/day)	Animal no	Sex	Days of study on which observed		
			Slight	Moderate or no degree indicated	Severe/tonic convulsions
40	3604	F	13		
200	4101	M	7 13	1	
200	4102	M		1	
200	4103	M	7 13	1 4 6	
200	4104	M	7 12 13	1	
200	4601	F	7	1	
200	4602	F		1	
200	4603	F	2 13 16 17	1	2 16 17
200	4604	F		4	

It is unclear why tremors were seen in the 1-year study but not in the 90-day study in the same species and at the same dose level. The DS did not consider them a clear indication of a neurotoxic effect as there are no indications of neurotoxicity in other studies and there were no other effects noted in this study that would support such a conclusion. However, the tremors are not considered a clearly non-specific effect caused by general toxicity (animals were relatively unaffected by dose at the beginning of the study) or stomach pain (the substance is not corrosive or irritating). However, if caused by the administration method (gelatine capsules), the tremors should have been observed in all dose groups including controls. In conclusion, the tremors seen were considered to be toxicologically relevant but the significance of the finding is unclear.

The DS proposed no classification for STOT SE.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

According to the CLP Regulation, specific target organ toxicity (single exposure) categories 1 and 2 is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture, which are not covered by the other hazard classes. Category 3 covers transient effects, occurring after single exposure, specifically respiratory tract irritation and narcotic effects.

Categories 1 and 2

In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. After inhalation exposure, clinical signs such as tremors, convulsions, low sensitivity, decreased motor activity, ptosis, ataxia and ventral position were observed at doses close to the LC_{50} value. According to the CLP guidance, care should be taken not to assign a "double classification" for the same effect and as these effects occurred close to the lethal doses, they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE. No adverse effects were noted in the acute neurotoxicity study (see the section on STOT RE for details). In the one-year dog study, tremors were seen shortly after dosing in a dose-response related manner during the initial three weeks of study but were not

observed subsequently. No tremors were observed in a 90-day study in the same species and at the same dose levels. The significance of the finding is unclear and in DS's opinion did not justify classification in STOT SE. There were no relevant early effects noted in the other repeated dose studies.

According to the results of the available studies, RAC agrees with the DS' proposal that thiophanate-methyl thereby does not fulfil the CLP criteria for STOT SE category 1 or 2.

Category 3

This hazard class is evaluated primarily on human data. No human data are available for thiophanate-methyl. However, appropriate animal data, e.g. clinical signs or histopathology data from acute inhalation studies can also be used, if available.
According to the CLP guidance section 3.8.2.3, clinical signs (e.g. dyspnoea, rhinitis etc.) and histopathology (hyperaemia, oedema, minimal inflammation, thickened mucous layer) observed in inhalation toxicity studies may justify classification for RTI and lethargy, lack of coordination, loss of righting reflex and ataxia observed in animal studies may justify classification for NE. Such effects were not seen in the studies available with thiophanate-methyl and classification in this category is therefore not justified.

RAC agrees with the DS', that **no classification as STOT SE is warranted** for thiophanate-methyl.

10.12 Specific target organ toxicity-repeated exposure

Table 57: Summary table of animal studies on STOT RE

Test substance **Route** **Duration of study**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Dose levels**	**Main findings**	**Reference**
Thiophanate-methyl Oral (dietary) 90-day Acceptable	Rat Fischer-344 M, F 10/sex/dose	0, 200, 2200, 4200, 6200 and 8200 ppm (equivalent to 0, 13.9, 155, 293.2, 426.9 and 564.7 mg/kg bw/day in males; 0, 15.7, 173.4, 323.0, 478.8 and 647.3 mg/kg bw/day in females)	<u>200 ppm:</u> No treatment related changes in males. - ↑ Thyroid weights in females (31%) <u>2200 ppm:</u> - ↑ Thyroid weights in males (39%) and females (50%) - ↑ Liver and kidney weights - Histopathological changes in thyroid (M, F), liver (M, F) and kidney (M) - Haematological changes (↓ Hb (-4%), - ↓ MCV, ↓ MCH, ↓ MCHC) - Effects on clinical chemistry (e.g. ↓ plasma Ch-E in females) <u>Notable effects at higher doses:</u> Findings at LOAEL increased in severity and incidence with increased dose. - ↓ Hb (-9%) at 8200 ppm. - ↑ T_3 in females at 8200 ppm - ↓ plasma Ch-E in females from 2200 ppm (>20%) - ↑ plasma Ch-E in males from 4200 ppm (>20%)	RAR Vol 3 B.6.3.2.1
Thiophanate-	Rat	0, 12.8, 64, 320, 1600	<u>8000 ppm:</u>	RAR Vol 3

methyl Oral (dietary) 90-day Supportive	Sprague-Dawley M, F 12/sex/dose	and 8000 ppm (equivalent to 0, 1, 4, 20, 95 and 500 mg/kg bw/day in males; 0, 1, 5, 22, 110 and 660 mg/kg bw/day in females)	- ↓ Bw (-28-30%) - Haematological changes (↓ Hct, ↓ RBC) - ↑ Relative liver (45-62%) and thyroid weights (51-62%) - Histopathological changes in thyroid	B.6.3.2.2
Thiophanate-methyl Subchronic neurotoxicity OECD 424 Oral (diet) 13 weeks Acceptable	Rat CD IGS M, F 10/sex/dose	0, 100, 500, and 2500 ppm (males: 6.2, 30.3 and 149.6 mg/kg bw/day, females: 6.8, 34.9 and 166.3 mg/kg bw/day)	<u>2500 ppm:</u> ↑ absolute liver weight (m: 20%) ↑ absolute thyroid weight (m: 100%) ↓ bw (f: -10%) ↓ food consumption (f)	RAR Vol 3 B.6.3.2.3
Thiophanate-methyl Oral (dietary) 90-day Supportive	Mouse ICR M, F 12/sex/dose	0, 12.8, 64, 320, 1600 and 8000 ppm (equivalent to 0, 2, 10, 50, 250 and 1240 mg/kg bw/day in males; 0, 2, 11, 52, 231, 1630 mg/kg bw/day in females)	<u>8000 ppm:</u> - ↓ Bw (-11-17%) - Haematological changes (↓ Hct, ↓ RBC) - ↑ Liver weight (17-18%) - Histopathological changes in liver	RAR Vol 3 B.6.3.2.5
Thiophanate-methyl Oral (capsule) 90-day Acceptable	Dog Beagle M, F 4/sex/dose	0, 50, 200 and 400 mg/kg bw/day (800 mg/kg bw/day for the first 7 weeks)	<u>50 mg/kg bw/day:</u> - histopathological changes (minimal follicular cell hypertrophy) in the thyroid (1m, 1f) <u>Notable effects at higher doses:</u> - One high dose male sacrificed in moribund condition at 800 mg/kg bw/day - ↓ Bw at 200 (-15-24%) and 400 mg/kg bw/day (-26-28%) - ↓ food consumption at 200 and 400 mg/kg bw/day - Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in females at 200 and 400 mg/kg bw/day and males at 400 mg/kg bw/day - Changes in biochemical parameters consistent with malnutrition and dehydration - ↓ T_3 and T_4 in females at 200 and 400 mg/kg bw/day and ↓ T_3 in males at 400 mg/kg bw/day - ↑ Liver and thyroid weights - Histopathological changes in the thyroid. Hypertrophy: incidence (0/8, 2/8, 5/8, 8/8) and severity increased with increased dose. Hyperplasia in 0/8, 0/8, 1/8, 7/8 at the respective dose levels.	RAR Vol 3 B.6.3.2.4
Thiophanate-methyl Oral (capsule)	Dog Beagle M, F 4/sex/dose	0, 8, 40 and 200 mg/kg bw/day	<u>8 mg/kg bw/day:</u> - ↑ Thyroid weight in females (29%) <u>Notable effects at higher doses:</u> -Tremors shortly after dosing at 200	RAR Vol 3 B.6.3.2.5

1 year Acceptable			mg/kg bw/day - ↓ Bw at 200 mg/kg bw/day (-20%) - Haematological changes (↓ Hct, ↓ Hb, ↓ RBC) in males at 200 mg/kg bw/day - ↓ T_4 in males at 200 mg/kg bw/day - Histopathological changes in the thyroid (hypertrophy and hyperplasia)	
Thiophanate-methyl Short-term Dermal 21 days Acceptable	Rabbit New-Zeeland White M, F 5/sex/dose	0, 100, 300 and 1000 mg/kg bw/day in males	No adverse effects were noted at any dose level.	RAR Vol 3 B.6.3.3.1
Thiophanate-methyl Developmental GD 6-19 Oral (gavage) Acceptable	Rat COBS CD 25 dams/dose	0, 100, 300 and 1000, mg/kg bw/day	<u>Maternal toxicity:</u> <u>1000 mg/kg bw/day:</u> ↓ adjusted bw gain (-22%) <u>Developmental toxicity:</u> No adverse effects	RAR Vol 3 B.6.6.2.1
Thiophanate-methyl Developmental GD 6-28 Oral (gavage) Supportive	Rabbit New Zealand white <u>Range-finding study:</u> 4 dams/dose <u>Main study:</u> 15 dams/dose	<u>Range-finding study:</u> 0, 10, 30, 100, 150, 300 and 600 mg/kg <u>Main study:</u> 0, 2, 6 and 20 mg/kg bw/day	**Range-finding study:** ↓ bw and food consumption at 30 mg/kg bw/day and above ↑ mortality at 150 mg/kg bw/day and above (1 at 150, 1 at 300, 2 at 600 mg/kg/bw/day) **Main study:** <u>Maternal toxicity:</u> <u>6 mg/kg bw/day:</u> ↓ bw GD 6-12 (-30 g compared to pre-dosing) <u>20 mg/kg bw/day:</u> ↓ bw GD 6-12 (-130 g compared to pre-dosing) <u>Developmental toxicity:</u> <u>6 and 20 mg/kg bw/day:</u> ↑ supernumerary ribs, thickened ribs, incomplete/asymmetrical ossification of costal elements of sacral vertebrae and asymmetric pelvis	RAR Vol 3 B.6.6.2.2
Thiophanate-methyl Developmental <u>Range-finding study:</u> GD 6-18 <u>Main study:</u> GD 6-28 <u>Range-finding</u>	Rabbit New Zealand white <u>Range-finding study:</u> 6 dams/dose <u>Main study:</u> 15 dams/dose	<u>Range-finding study:</u> 0, 5, 10, 20, 40 and 80 mg/kg/day <u>Main study:</u> 0, 5, 10, 20 and 40 mg/kg/day	**Range-finding study:** <u>Maternal toxicity:</u> <u>40 mg/kg bw/day:</u> ↑ abortions (3/6 does) (gavage group) ↓ bw and food consumption <u>80 mg/kg bw/day:</u> ↑ abortions (3/6 does) (gavage group) ↓ bw and food consumption <u>Developmental toxicity:</u> <u>80 mg/kg bw/day:</u> ↑ resorptions (gavage group) ↑ number of thoracic vertebrae	RAR Vol 3 B.6.6.2.3

study: Oral (gavage) and dietary Main study: Oral (gavage) Acceptable			↓ number of lumbar vertebrae ↑ number of rib pairs **Main study:** Maternal toxicity: 20 mg/kg bw/day: ↓ bw gain (-12%) compared to controls ↓ food consumption 40 mg/kg bw/day: ↓ bw gain (-76%) compared to controls ↓ food consumption ↓ faecal output Developmental toxicity: 40 mg/kg bw/day: ↑ supernumerary thoracic ribs	

Table 58: Summary table of human data on STOT RE

No data.

Table 59: Summary table of other studies relevant for STOT RE

No data.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

All studies are thoroughly presented in the Annex I(Volume 3 to the RAR).

Two short-term studies with thiophanate-methyl are available with rats. In the first study, considered as acceptable, haematology effects (reduced Hb (-4%), MCV, MCH and MCHC) and dose-related histopathological effects, accompanied by weight changes of the affected organs, were observed in the thyroid, liver, adrenal and kidney at 2200 ppm and above. There was also an increase in total cholesterol, total protein, albumin and calcium in animals at 2200 ppm and above as well as increased T3 levels noted in males at 8200 ppm. There was also a statistically significant ($p<0.01$) and dose-related decrease in plasma cholinesterase starting from 2200 ppm in females and increase from 4200 ppm in males.

The second rat study was limited in scope and reporting and is considered to provide supportive information only. Effects were seen at the highest dose of 8000 ppm (500-660 mg/kg bw/day in males-females) and consisted of reduced body weights (28-30%), slight effects on haematology (reduced Hct by 4-10% and RBC count by 10-14%) and increased thyroid weights (absolute: 12% in males and 18% in females) accompanied by histopathological changes (follicles, cubic epithelium cells and a decrease of colloidal substance).

The neurotoxicity potential of thiophanate-methyl was investigated after subchronic exposure to rats. No neurotoxic effect was observed. However, in line with the results obtained in previous studies, reduced body weight/body weight gain and food consumption as well as increased liver (m: 20%) and thyroid weights (m: 100%) were noted at 2500 ppm.

In a mouse study, also considered to provide supportive information, treatment with thiophanate-methyl was associated with reduced bodyweights (-11-17%), slight effects on haematology (reduced Hct by 8-13% and RBC count by 7-12%) and increased liver weights accompanied by hepatocyte swelling and enlargement (hypertrophy) at 8000 ppm (1240-1630 mg/kg bw/day in males-females).

Two studies with thiophanate-methyl are available with dogs. In the 90-day study, treatment with thiophanate-methyl was associated with severe weight loss leading to the sacrifice of one dog at the highest dose (800 mg/kg bw/day) and lowering of the dose to 400 mg/kg bw/day from week 8 onwards. Evaluation of haematology and biochemistry values revealed changes consistent with malnutrition/dehydration in mid-

and high-dose dogs. Reductions in Hb (-12-25%) and Hct (-10-24%) together with increases in platelet count and Activated Partial Thromboplastin Time were noted at 400 mg/kg bw/day with Hb also reduced by 11% in females at 200 mg/kg bw/day (all statistically significant). T3 levels were reduced in females at both 200 and 400 mg/kg bw/day. In spite of marked decreases in terminal body weights, absolute liver (6-11%) and thyroid weights (11-49%) were increased compared to concurrent controls. Statistical significance was however reached for relative weights only. Hypertrophy of the follicular epithelial cells of the thyroid was seen in each treated group, but not in the control group. Minimal to marked hyperplasia of the follicular epithelium was also seen starting from 200 mg/kg bw/day.

In the one-year dog study, tremors were seen shortly after dosing on one or more occasions during the initial three weeks of study but were not observed subsequently. Reduced body weights (-20%) and slight decreases in Hb, Hct and RBC (in males only) were also observed as well as alterations in biochemistry. There was a reduction in T4 levels in males but no effects on T3 or TSH in any sex at any dose level. As in the subchronic dog study, absolute liver and thyroid weights were increased compared to concurrent controls but statistical significance was reached for relative weights only. Minimal to moderate hypertrophy and slight hyperplasia was also noted in the thyroid.

In the 90-day dermal toxicity study in rabbits, the only effect observed was a reduction in food consumption at the highest dose of 1000 mg/kg bw/day.

Studies relevant for STOT RE classification also include chronic toxicity studies and reproductive toxicity studies (section 10.10).

The findings in the long-term toxicity studies (see section 10.9) were consistent with results of subchronic studies identifying the liver, kidney and the thyroid as target organs also following long-term exposure. In addition, thiophanate-methyl was shown to induce anaemia in the rat.

The findings in the reproductive toxicity studies (see section 10.10) were also consistent with results of subchronic studies identifying the liver and thyroid as target organs. Mortality was noted in one developmental toxicity study (RAR Vol 3 B.6.2.2.2), see below.

10.12.2 Comparison with the CLP criteria

Regulation (EC) No 1272/2008 (CLP), 3.9.2.7.3, states for STOT RE:

> *"All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:*
>
> *(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;*
>
> *(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);*
>
> *(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;*
>
> *(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;*
>
> *(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;*
>
> *(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);*

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

Effects noted in studies with repeated exposure to thiophanate-methyl considered to be potentially relevant in terms of classification include mortality, haematological effects and effects on thyroid.

Mortality

Mortality was noted in dogs and rabbits. In dogs, mortality was seen in a 90-day study (RAR Vol 3, B.6.3.4.2) at 800 mg/kg bw/day. This is above the Guidance Value for STOT RE 2 of 100 mg/kg bw/day for a 90-day study and not considered relevant for classification.

In rabbits, mortality was observed at 150 (1 animal), 300 (1 animal) and 600 (2 animals) mg/kg bw/day in a developmental toxicity study (RAR Vol 3 B.6.2.2.2). Exposure in this study was for 14 days (GDs 6-19) and mortality occurred on days 19 (150 mg/kg bw/day), 24 (300 mg/kg bw/day), 20 and 23 (600 mg/kg bw/day). It is therefore considered an effect of repeated administration of the test substance rather than an acute effect. As exposure was for 14 days *i.e.* 1/6 of 90 days, it is proposed to multiply the Guidance Values of 10 for Cat 1 and 100 for Cat 2 classification of 10 and 100 mg/kg bw/day, respectively, by a factor 6, giving adjusted Guidance Values for 14 days of 60 and 600 mg/kg bw/day for STOT RE 1 and 2, respectively. The mortalities occurred above 60 mg/kg bw/day but below 600 mg/kg bw/day and therefore justify classification in STOT RE 2.

Haematological effects

Haematological effects were seen in both rats and dogs. In the 90-day study in dogs, Hb was reduced by 25% in males and 12% in females at 400 mg/kg bw/day and by 11% in females at 200 mg/kg bw/day, which is above the Guidance Value of 100 mg/kg bw/day for a 90-day study. There were also reductions in Htc, APTT and platelet count at the higher dose level but no other signs of anaemia observed in the study.

The effect was less pronounced in the 1-year dog study with Hb reduced by 14% in males at 12 months at 200 mg/kg bw/day but no reduction observed in females, and again above the Guidance Value.

Reductions in Hb, MCV, MCH, MCHC and PCV were also observed in the 90-day study in rats, but these reductions were of low magnitude (<10%) and were not accompanied of other effects indicative of anaemia, thus not justifying classification. In the 2-year study in rats, treatment-related anaemia (decreases of red blood cell count, haemoglobin, haematocrit, MCV, MCH and MCHC) was noted in both sexes at 300 mg/kg bw/day but not to any significant degree at lower dose levels. As these findings were above the Guidance Value, they also do not justify classification.

Thyroid

Effects on thyroid were seen in rats and dogs in studies with repeated administration of thiophanate-methyl. Slight effects were also seen in the 2-year mouse study. The effects are usually first seen as hypertrophy which then progresses to hyperplasia and in the 2-year study in rats then to adenoma and in a few animals to carcinoma (albeit at doses exceeding the MTD). Effects on T4, T3 and TSH were also seen, see the table below.

Table 60: Summary of *in vivo* data referring to findings in liver and thyroid (effects marked with bold indicate doses within the Guidance value for classification in STOT RE 2)

Study	Species	Dose	Liver effects	Thyroid effects
90-day study	Rat RAR Vol 3, B.6.3.2.1	*Guidance value STOT RE 2: 100 mg/kg bw/day*		
		200 ppm (M: 13.9 mg/kg bw/d; F:15.7 mg/kg bw/d)	No effects	**F: Weight↑**

		2200 ppm (M: 155.0 mg/kg bw/d; F: 173.4 mg/kg bw/d)	F: Weight↑ M, F: hypertrophy	M, F: Weight ↑, M, F: Hypertrophy/hyperplasia
		4200 ppm (M: 293.2 mg/kg bw/d; F: 323.0 mg/kg bw/d)	M, F: Weight ↑ M, F: hypertrophy	
		6200 ppm (M: 426.9 mg/kg bw/d; F: 478.8 mg/kg bw/d)		
		8200 ppm (M: 564.7 mg/kg bw/d; F: 647.3 mg/kg bw/d)		
	Dog RAR Vol 3, B.6.3.2.4	*Guidance value STOT RE 2: 100 mg/kg bw/day*		
		50 mg/kg bw/d	No effects	**M, F: hypertrophy; hyperplasia 2 animals – minimal severity**
		200 mg/kg bw/d	M, F: Weight↑	F: T3, T4↓ M: Weight↑ M, F: hypertrophy or hyperplasia
		800/400 mg/kg bw/d		M: T3↓, F: T3, T4↓ M: Weight↑ M, F: hypertrophy or hyperplasia
Long-term toxicity study	Rat 2 year RAR Vol 3, B.6.5.1	*Guidance value STOT RE 2: 12.5 mg/kg bw/day*		
		75 ppm (M: 3.3 mg/kg bw/d; F: 3.8 mg/kg bw/d)	No effects	**No effects**
		200 ppm (M: 8.8 mg/kg bw/d; F: 10.2 mg/kg bw/d)	No effects	**No effects**
		1200 ppm (M: 54.4 mg/kg bw/d; F: 63.5 mg/kg bw/d)	M, F: Weight ↑ M, F: hypertrophy	M, F: Weight ↑ M, F: hypertrophy/hyperplasia
		6000 ppm (M: 280.6 mg/kg bw/d; F: 334.7 mg/kg bw/d)		M, F: Weight ↑ M, F: hypertrophy/hyperplasia, adenoma
	Dog 1 year RAR Vol 3, B.6.3.2.5	*Guidance value STOT RE 2: 25 mg/kg bw/day*		
		8 mg/kg bw/d	No effects	**F: Weight↑**
		40 mg/kg bw/d	No effects	M, F: Weight↑ F: Hypertrophy, (M: T4↓, interim: 6 m)
		200 mg/kg bw/d	M, F: Weight ↑	M, F: Weight↑ M, F: Hypertrophy; hyperplasia (2 animals) (M: T4↓, interim 6 m and 12 m)
	Mouse 18-m RAR Vol 3, B.6.5.3	Guidance value STOT RE 2: 16.7 mg/kg bw/day		
		150 ppm (M: 23.7 mg/kg bw/d, F: 28.7 mg/kg bw/d)	No effects	No effects
		640 ppm (M: 98.6 mg/kg bw/d, F: 123.3 mg/kg bw/d)	F: hypertrophy	No effects
		3000 ppm (M: 467.6 mg/kg bw/d,	M,F: Weight ↑ M, F hypertrophy,	M: Weight ↑ TSH ↑ at 9 m but not at 18

		F: 557.9 mg/kg bw/d)	adenoma	m
		7000 ppm (M: 1078.8 mg/kg bw/d, F: 1329.4 mg/kg bw/d)	M, F: Weight ↑ M, F hypertrophy, adenoma	M, F: Weight ↑ TSH ↑ at 9 m but not at 18 m F. T4 ↓ at 9 m but not at 18 m
Mechanism study (in vivo)	Rat RAR Vol 3, B.6.5.1	6000 ppm Male	Weight↑ T-Cho↑ P-450, UDP-GT↑ PCNA↑, TPO ↓	Weight ↑ T3, T4 ↓ , TSH ↑
2-gen study	Rat RAR Vol 3, B.6.6.1.1	*Guidance value STOT RE 2: 40 mg/kg bw/day*		
		200 ppm (M: 14.6 mg/kg bw/d, F: 18.0 mg/kg bw/d)	M, F: hypertrophy	**(F: Weight)** **M : hypertrophy /hyperplasia**
		630 ppm (M: 46.0 mg/kg bw/d, F: 55.4 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy	M, F: Weight↑ M, F: hypertrophy/hyperplasia
		2000 ppm (M: 147.1 mg/kg bw/d, F: 172.9 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy	M, F: Weight↑ M, F: hypertrophy/hyperplasia M, F: TSH ↑ , M: T4 ↓
90-day neurotoxicity study	Rat RAR Vol 3, B.6.7.1.2	*Guidance value STOT RE 2: 100 mg/kg bw/day*		
		100 ppm (M: 6.2 mg/kg bw/d, F: 6.8 mg/kg bw/d)	No effects (histopathology not investigated)	**No effects (histopathology and hormones not investigated)**
		500 ppm (M: 30.3 mg/kg bw/d, F: 34.9 mg/kg bw/d)		
		2500 ppm (M: 149.6 mg/kg bw/d, F: 166.3 mg/kg bw/d)	M: Weight↑ (histopathology not investigated)	M, F: Weight↑ (histopathology and hormones not investigated)

In addition to the thyroid effects there was also an effect on liver, as shown in the table. The mechanistic study (RAR Vol 3, B.6.5.1) showed that there was an upregulation of uridine diphospho-glucuronosyltransferase (UDPGT), indicating that the thyroid effects could a result of increased hepatic clearance of T4. Rodents (especially the rat) are generally considered more sensitive to this effect than humans.

However, the mechanistic study also showed that thiophanate-methyl inhibited thyroid peroxidase (TPO) which oxidizes iodide ions in the thyroid to form iodine atoms for addition onto tyrosine residues on thyroglobulin for the production of T4 or T3. The study also showed that T4 supplementation had no influence on the increased liver weight and that phenobarbital, which was used as a reference substance, also induced liver cell proliferation and the drug metabolizing enzymes but caused only a very slight increase of TSH without thyroid hypertrophy, further indicating that the increased UDPGT was not the only mechanism behind the thyroid effects. Thus, it was concluded in the study that the inhibition of hormone synthesis in the thyroid due to an inhibition of TPO could be the main cause of the T4 depression.

In March 2017 a workshop was held at ANSES (FR) and the draft report from the workshop[5] provides in-depth information about the thyroid system, including differences between species and relevance of animal models to humans, case studies of thyroid-disrupting substances and recommendations on data interpretation and evaluation. It is concluded in the draft report is that a mode of action that involves upregulation of UDPGT is likely to be more relevant to humans than previously thought. There is also a case study of

[5] Supporting the organisation of a workshop on thyroid disruption – Draft Final Report. December 2016

mancozeb where the mode of action was TPO inhibition. Thyroid effects were seen in all tested species (rat, mouse, dog and monkey) and it was concluded that this fact indicated that "the mechanism of TPO inhibition is probably also a relevant molecular initiating event in humans".

The thyroid effects seen in the studies are therefore considered relevant for the purpose of classification.

The endocrine properties of thiophanate-methyl were discussed at EFSA's PPR 162 meeting. All experts were of the opinion that thiophanate-methyl should be considered to be an endocrine disruptor (however not according to the interim criteria as laid out in Regulation (EC) No 1107/2009 Annex II point 3.6.5) and that the effects in the thyroid should be regarded as relevant for humans.

Comparison with Guidance values for classification in STOT RE 2In the 90-day rat study, thyroid weights were increased in females by 31% compared to controls (statistically significant) at 16 mg/kg bw/day. At the next dose of 155 mg/kg bw/day and above, thyroid weights were increased in both sexes in a dose-related manner and the finding was accompanied by follicular hyperplasia and hypertrophy. Only the 31% weight increase in females was within the Guidance Value of 100 mg/kg bw/day for a 90-day study.

In the 2-generation study in rats (RAR Vol 3, B.6.6.1.1), the test substance was given continuously from the start of treatment until necropsy. Based on these data, information on exposure time for the P and F1 generation is given in the table below.

	Days of study		
	P generation	F1 generation (first mating)	F1 generation (second mating)
Males	227 – 240	165	143 – 157
Females	241 – 249	165	163 – 165

Exposure was for maximum 240 days in males (the sex where most effects were seen), and a Guidance Value of approximately 40 mg/kg bw/day can then be calculated (90 / 240 = 0.375. 100 mg/kg bw/day x 0.375 = 37.5 mg/kg bw/day). The following effects were observed below this dose level (200 ppm or 15-18 mg/kg bw/day): Thyroid hypertrophy was observed in 9/25 males at in the P generation and 3/25 males of the F1 generation. Incidences were lower in females with 1/24 females of the P generation and 0/24 in the F1 generation being affected. Thyroid hyperplasia in males was observed in 4-6/25 (study author-peer reviewer) animals the P generation and 4-1/25 (study author-peer reviewer) animals in the F1 generation at the same dose level. There was no hyperplasia noted in females at this dose, however thyroid weights were statistically significantly increased (9%). At doses above the Guidance Value, higher incidences of thyroid hypertrophy and hyperplasia were observed.

In a 2-year study in rats (RAR Vol 3, B.6.5.1), increased thyroid weights (m: 24%, f: 27%), morphological and functional changes in the thyroid and follicular cell hyperplasia and hypertrophy (m: 38%, f: 47%) were observed at 1200 ppm (54.4 and 63.5 mg/kg bw/day in male and female rats, respectively). This dose level is above the Guidance Value for a 2-year rat study of 12.5 mg/kg bw/day do therefore not justify classification in STOT RE 2.

In the 90-day dog study (RAR Vol 3, B.6.3.2.4), histopathological changes (minimal follicular cell hypertrophy) in the thyroid (1m, 1f) were observed at 50 mg/kg bw/day. At the next dose of 200 mg/kg/bw/day (above the Guidance Value for a 90-day study) this finding increased in severity and incidence and hyperplasia and increased organ weight was observed as well as a reduction in T3 and T4.

In the 1-year dog study, thyroid weights were increased by 29% in females at 8 mg/kg bw/day (within the Guidance Value). Microscopic alterations attributed to test material administration were limited to the follicular epithelium of the thyroid gland with minimal to moderate hypertrophy in males (0/0, 0/0, 0/0, 4/4) and females (0/0, 0/0, 2/4, 3/4) and slight hyperplasia (group IV, one male and one female). These effects were noted above the Guidance Value.

Table 61: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
RAR Vol 3B.6.2.2.2	150-600	14 d	25-100	Yes
RAR Vol 3, B.6.6.1.1	15-18	240 d	40-48	Yes
RAR Vol 3, B.6.5.1	54.4 - 63.5	2 years	Approx. 440	No

10.12.3 Conclusion on classification and labelling for STOT RE

Classification in **STOT RE 2** is proposed based on the mortalities seen in rabbits at 125, 300 and 600 mg/kg bw/day in a developmental toxicity study with 14 days exposure to thiophanate-methyl, as well as on thyroid effects (hypertrophy, hyperplasia and increased organ weight) in rats, supported by similar findings indogs. The following hazard statement is proposed: **H373 – May cause damage to organs through prolonged or repeated exposure**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Several repeated studies are present in the CLH dossier on thiophanate-methyl.

Four of these are on rat. In the first 90 days study, thiophanate-methyl was administered orally in the diet at 0, 13.9, 155, 293.2, 426.9 and 564.7 mg/kg bw/d in males; 0, 15.7, 173.4, 323.0, 478.8 and 647.3 mg/kg bw/d in females on Fischer-344 rats. The haematology effects consisted in reduced Hb, MCV, MCH and MCHC and dose-related histopathological effects and were observed in the thyroid, liver, adrenal and kidney at 155-173.4 mg/kg bw/d (m-f, respectively) and above, accompanied by weight changes of the affected organs. There was also an increase in total cholesterol, total protein, albumin and calcium in animals at the same dose and above as well as increased T3 levels noted in males at 564.7 mg/kg bw/d. There was also a statistically significant ($p < 0.01$) and dose-related decrease in plasma cholinesterase starting from 155 mg/kg bw/d in females and increase from 323 mg/kg bw/d in males.

In the second 90 days study (considered supportive due to limitation in scope and reporting), thiophanate-methyl was administered orally in the diet at 0, 1, 4, 20, 95 and 500 mg/kg bw/d in males; 0, 1, 5, 22, 110 and 660 mg/kg bw/d in females on Sprague-Dawley rats. Effects were seen at the highest dose of 500-660 mg/kg bw/d in males-females respectively and consisted of reduced body weights (28-30 %), slight effects on haematology (reduced haematocrit by 4-10 % and RBC count by 10-14 %) and increased thyroid weights (absolute: 12 % in males and 18 % in females) accompanied by histopathological changes (follicles, cubic epithelium cells and a decrease of colloidal

substance).

The third study was conducted on CD IGS rats. Thiophanate-methyl was administered orally in the diet at 0 6.2, 30.3 and 149.6 mg/kg bw/d in male and 6.8, 34.9 and 166.3 mg/kg bw/d in females for 13 weeks. The study was performed to investigate neurotoxicity following the OECD TG 424. Although no neurotoxic effects have been found, the findings are in line with the results obtained in previous studies, i.e. reduced body weight/body weight gain and food consumption as well as increased liver (in male 20%) and thyroid weights (in male 100 %) were noted at 149.6 mg/kg bw/d in male and 166.3 mg/kg bw/d in females.

In the CLH report, (section 10.12) a developmental study was submitted on COBS CD rats administrated by gavage at 0, 100, 300 and 1 000, mg/kg bw/d on GD 9-16. The study showed maternal toxicity at the highest dose consisting in reduced bw gain (-22 %).

One 90 days study was submitted on mouse (ICR). Thiophanate-methyl was administered orally in the diet at 0, 12.8, 64, 320, 1 600 and 8 000 ppm (equivalent to 0, 2, 10, 50, 250 and 1 240 mg/kg bw/d in males; 0, 2, 11, 52, 231, 1 630 mg/kg bw/d in females). The study provided supportive information. Treatment with thiophanate-methyl was associated with reduced bodyweights (-11-17 %), slight effects on haematology (reduced Hct by 8-13 % and RBC count by 7-12 %) and increased liver weights accompanied by hepatocyte swelling and enlargement (hypertrophy) at 8 000 ppm (1 240-1 630 mg/kg bw/d in males-females, respectively).

In the 90-day dermal toxicity study in rabbits, the only effect observed was a reduction in food consumption at the highest dose of 1 000 mg/kg bw/d.

Two repeated studies with thiophanate-methyl are available with dogs (Beagle). In the 90 day oral (in capsule) study, treatment with thiophanate-methyl at 0, 50, 200 and 400 mg/kg bw/d (800 mg/kg bw/d for the first 7 weeks) was associated with severe weight loss leading to the sacrifice of one dog at the highest dose (800 mg/kg bw/d) and lowering of the dose to 400 mg/kg bw/d from week 8 onwards. Evaluation of haematology and biochemistry values revealed changes consistent with malnutrition/dehydration in mid- and high-dose dogs. Reductions in Hb (-12-25 %) and Hct (-10-24 %) together with increases in platelet count and Activated Partial Thromboplastin Time were noted at 400 mg/kg bw/d with Hb also reduced by 11 % in females at 200 mg/kg bw/d (all statistically significant). T3 levels were reduced in females at both 200 and 400 mg/kg bw/d. In spite of marked decreases in terminal body weights, absolute liver (6-11 %) and thyroid weights (11-49 %) were increased compared to concurrent controls. Statistical significance was however reached for relative weights only. Hypertrophy of the follicular epithelial cells of the thyroid was seen in each treated group, but not in the control group. Minimal to marked hyperplasia of the follicular epithelium was also seen starting from 200 mg/kg bw/d.

In the one-year oral (in capsule) dog study at 0, 8, 40 and 200 mg/kg bw/d with thiophanate-methyl, tremors were seen shortly after dosing on one or more occasions during the initial three weeks of study but were not observed subsequently. Reduced body weights (-20 %) and slight decreases in Hb, haematocrit and RBC (in males only) were also observed as well as alterations in biochemistry. There was a reduction in T4 levels in males but no effects on T3 or TSH in any sex at any dose level. As in the 90 day

dog study, absolute liver and thyroid weights were increased compared to concurrent controls but statistical significance was reached for relative weights only. Minimal to moderate hypertrophy and slight hyperplasia was also noted in the thyroid.

In an oral (in diet) 2-generation reproduction study conducted on Rat (Sprague-Dawley Crl:CD (SD)BR) following the OECD TG 416 with thiophanate-methyl at 0, 14.6, 46.0 and 147.1 mg/kg bw/d in males; 0, 18.0, 55.4 and 172.9 mg/kg bw/d in females the following effects were observed: at 18.0 were seen an increase in thyroid weight in female (18 %) and histopathological changes in the thyroid (hypertrophy, hyperplasia) and liver (hypertrophy). Notable effects at higher doses were increases in thyroid and liver weights and hepatocyte hypertrophy and thyroid hypertrophy at 55 and 172.9 mg/kg bw/d and increase in TSH levels at 172.9 mg/kg bw/d, decrease in T4 levels and reduced body weight gain at 147.1 mg/kg bw/d (in males). In the offspring has been noticed at 18 mg/kg bw/d reduced body weight (-9 %) and effects at higher doses were reduced body weight (-10-14 % at 55.4 mg/kg bw/d; -13-16 % at 172.9 mg/kg bw/d).

In a combined chronic/oncogenicity study in rats following the OECD TG 453 with deviation (fully described in the Carcinogenicity section RAR Vol 3 B.6.5.1) effects on the thyroid at the two high doses were considered by the DS to be related to the changes in hormonal homeostasis of the pituitary-thyroid axis. The continuous stimulation of the thyroid gland by TSH due to reductions in T4/T3 is known to result in follicular cell hypertrophy/hyperplasia and depending on dose and time in follicular cell adenomas/adenocarcinomas.

In rabbits, mortality was observed at 150 (1 animal), 300 (1 animal) and 600 (2 animals) mg/kg bw/d in a developmental toxicity study (RAR Vol 3 B.6.2.2.2). Exposure in this study was for 14 days (GDs 6-19) and mortality occurred on days 19 (150 mg/kg bw/d), 24 (300 mg/kg bw/d), 20 and 23 (600 mg/kg bw/d). It was therefore considered an effect of repeated administration of the test substance rather than an acute effect.

Classification in STOT RE 2 was proposed by DS based on the mortalities seen in rabbits at 125, 300 and 600 mg/kg bw/d in a developmental toxicity study with 14 days exposure to thiophanate-methyl, as well as on thyroid effects (hypertrophy, hyperplasia and increased organ weight) in rats, supported by similar findings in dogs. The following hazard statement was proposed: H373 – May cause damage to organs through prolonged or repeated exposure.

Comments received during public consultation

Three comments by MSCA were received during the public consultation, 2 in favour and one against the classification as STOT RE 2.

The two MSCA supported the classification as STOT RE 2 based on the data presented (mortalities in pregnant rabbit and thyroid effects) and proposed to specify the respective organ (thyroid) in the hazard statement of classification:”H373: May cause damage to the thyroid through prolonged or repeated exposure”. The DS agreed with this proposal.

The comment against the classification was based on that, even if the thyroid size is increased in a clearly dose dependent manner at or above the guidance value for STOT RE 2, a mere increase in organ size (hypertrophy/hyperplasia) is not an adverse effect sufficient to warrant classification for STOT RE. Moreover this MSCA stated that the

criteria allow classification when a relevant mechanism is demonstrated or relevant secondary effects or relevant organ dysfunctions are demonstrated. However in this case, thiophanate-methyl inhibits TPO resulting in reduced thyroid hormone levels that has an effect on thyroid size. It is thus considered by the MSCA that the thyroid size is a result from thyroid hormone inhibition and that a mere increased thyroid size can be seen as a successful adaptive mechanism without further toxicological relevant consequence. Notably, the mortality observed starting at 150 mg/kg bw/d in the rabbit developmental toxicity range finding study (dosed for 14 days) does not seem as relevant for classification for STOT RE due to low mortality incidence without a clear dose/time-dependent relationship. Due to the short duration of developmental tests, the MSCA also states that it is unclear whether this should be considered acute toxicity or repeated dose toxicity.

Assessment and comparison with the classification criteria

All the studies relevant in terms of classification including mortality, haematological effects and effects on thyroid and effects have been evaluated to consider the effects of repeated exposure to thiophanate-methyl.

Mortality was noted both in dogs and in rabbits. In dogs, mortality was seen in a 90-day study (RAR Vol 3, B.6.3.4.2) at 800 mg/kg bw/d. This is above the Guidance Value for STOT RE 2 of 100 mg/kg bw/d for a 90-day study and not considered relevant for classification.

In rabbits, mortality was observed at 150 (1 animal), 300 (1 animal) and 600 (2 animals) mg/kg bw/d in a developmental toxicity study (RAR Vol 3 B.6.2.2.2). Exposure in this study was for 14 days at GDs 6-19. Mortality occurred on days 19 (150 mg/kg bw/d), 24 (300 mg/kg bw/d), 20 and 23 (600 mg/kg bw/d) and it is considered an effect of the repeated administration of the test substance. As exposure was for 14 days i.e. 1/6 of 90 days, the Guidance Values of 10 mg/kg bw/d for Cat. 1 and 100 mg/kg bw/d for Cat. 2 classification should be multiplied by a factor 6, giving adjusted Guidance Values for 14 days of 60 and 600 mg/kg bw/d for STOT RE 1 and 2, respectively. As the mortalities occurred above 60 mg/kg bw/d but below 600 mg/kg bw/d, classification in STOT RE 2 may be justified. However it should be noted that in this study all females showed a decline in general health and condition with an associated reduction food intake, pronounced body weight loss and reduced faeces, therefore mortality was observed at dose levels exceeding the MTD.

Haematological effects were seen in rats and dogs but, for both the findings were above the Guidance Value and do not justify classification.

Effects on thyroid were seen in rats and dogs in studies with repeated administration of thiophanate-methyl. Slight effects were also seen in the 2-year mouse study. The effects were usually first seen as hypertrophy which then progressed to hyperplasia and in the 2-year study in rats then to adenoma and in a few animals to carcinoma (albeit at doses exceeding the MTD). Effects on T4, T3 and TSH were also reported.

In the table below a summary of *in vivo* data referring to findings in thyroid is shown (effects marked with bold indicate doses within the Guidance value for classification in STOT RE 2).

Table: *summary of in vivo data referring to findings in thyroid*

Study	Species	Dose	Thyroid effects
90-day study	Rat RAR Vol 3, B.6.3.2.1	Guidance value STOT RE 2: 100 mg/kg bw/d	
		200 ppm (M: 13.9 mg/kg bw/d; F:15.7 mg/kg bw/d)	**F: Weight**↑
		2 200 ppm (M: 155.0 mg/kg bw/d; F: 173.4 mg/kg bw/d)	M, F: Weight ↑, M, F: Hypertrophy/hyperplasia
		4 200 ppm (M: 293.2 mg/kg bw/d; F: 323.0 mg/kg bw/d)	
		6 200 ppm (M: 426.9 mg/kg bw/d; F: 478.8 mg/kg bw/d)	
		8 200 ppm (M: 564.7 mg/kg bw/d; F: 647.3 mg/kg bw/d)	
	Dog RAR Vol 3, B.6.3.2.4	Guidance value STOT RE 2: 100 mg/kg bw/d	
		50 mg/kg bw/d	**M, F: hypertrophy; 2 animals – minimal severity**
		200 mg/kg bw/d	F: T3, T4↓ M: Weight↑ M, F: hypertrophy or hyperplasia
		800/400 mg/kg bw/d	M: T3↓, F: T3, T4↓ M: Weight↑ M, F: hypertrophy or hyperplasia
Long-term toxicity study	Rat 2 year RAR Vol 3, B.6.5.1	Guidance value STOT RE 2: 12.5 mg/kg bw/d	
		75 ppm (M: 3.3 mg/kg bw/d; F: 3.8 mg/kg bw/d)	No effects
		200 ppm (M: 8.8 mg/kg bw/d; F: 10.2 mg/kg bw/d)	No effects
		1 200 ppm (M: 54.4 mg/kg bw/d; F: 63.5 mg/kg bw/d)	M, F: Weight ↑ M, F: hypertrophy/hyperplasia

		6 000 ppm (M: 280.6 mg/kg bw/d; F: 334.7 mg/kg bw/d)	M, F: Weight ↑ M, F: hypertrophy/hyperplasia, adenoma
	Dog 1 year RAR Vol 3, B.6.3.2.5	Guidance value STOT RE 2: 25 mg/kg bw/d	
		8 mg/kg bw/d	F: Weight↑
		40 mg/kg bw/d	M, F: Weight↑ F: Hypertrophy, (M: T4↓, interim: 6 m)
		200 mg/kg bw/d	M, F: Weight↑ M, F: Hypertrophy; hyperplasia (2 animals) (M: T4↓, interim 6 m and 12 m)
	Mouse 18-m RAR Vol 3, B.6.5.3	Guidance value STOT RE 2: 16.7 mg/kg bw/d	
		150 ppm (M: 23.7 mg/kg bw/d, F: 28.7 mg/kg bw/d)	No effects
		640 ppm (M: 98.6 mg/kg bw/d, F: 123.3 mg/kg bw/d)	No effects
		3 000 ppm (M: 467.6 mg/kg bw/d, F: 557.9 mg/kg bw/d)	M: Weight ↑ TSH ↑at 9 m but not at 18 m
		7 000 ppm (M: 1078.8 mg/kg bw/d, F: 1 329.4 mg/kg bw/d)	M, F: Weight ↑ TSH ↑at 9 m but not at 18 m F. T4↓at 9 m but not at 18 m
Mechanism study (*in vivo*)	Rat RAR Vol 3, B.6.5.1	6 000 ppm Male	Weight ↑ T3, T4↓, TSH↑
2-gen study	Rat RAR Vol 3, B.6.6.1.1	Guidance value STOT RE 2: 40 mg/kg bw/d	
		200 ppm (M: 14.6 mg/kg bw/d, F: 18.0 mg/kg bw/d)	**(F: Weight)** **M : hypertrophy /hyperplasia**
		630 ppm (M: 46.0 mg/kg bw/d, F: 55.4 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy/hyperplasia
		2 000 ppm (M: 147.1 mg/kg bw/d, F: 172.9 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy/hyperplasia M, F: TSH↑, M: T4↓
90-day neurotoxicity study	Rat RAR Vol 3, B.6.7.1.2	Guidance value STOT RE 2: 100 mg/kg bw/d	
		100 ppm (M: 6.2 mg/kg bw/d, F: 6.8 mg/kg bw/d)	No effects (histopathology and hormones not investigated)
		500 ppm (M: 30.3 mg/kg bw/d, F: 34.9 mg/kg bw/d)	
		2 500 ppm (M: 149.6 mg/kg bw/d, F:	M, F: Weight↑ (histopathology and

		166.3 mg/kg bw/d)	hormones not investigated)

Whereas thyroid is clearly a target organ of thiophanate-methyl toxicity, it is noted that in none of the studies effects indicative of significant or severe toxicity were observed at dose levels within the guidance values for classification. At dose levels at or below the guidance value there were either no effects (most studies), or effects on thyroid weight without histopathological findings (90-d rat, 1-yr dog). Only in two studies some histopathological findings were observed. In the 90-d dog study it concerned minimal hypertrophy in 2/8 dogs, in the 2-gen rat study hypertrophy and hyperplasia (no severity score reported). At comparable doses in a 3-gen rat study (RAR Vol 3, B.6.6.1.2) these histopathological changes were however not observed. Overall these effects were observed at dose levels below the guidance value, possibly indicating an adaptive response, and are not sufficient to warrant classification.

In conclusion, the mortality observed in rabbits in a developmental toxicity study and the effects on the thyroid (increased weight, hypertrophy and hyperplasia) in rats and dogs observed upon short- to long-term exposure were considered by the RAC not sufficient to support the classification of thiophanate-methyl as STOT RE 2. Therefore **no classification is proposed.**

10.13 Aspiration hazard

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

No data is available.

10.13.2 Comparison with the CLP criteria

Not relevant as no data is available.

10.13.3 Conclusion on classification and labelling for aspiration hazard

No classification is proposed for thiophanate-methyl.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

All the information on rapid biodegradability are taken from the RAR for thiophanate-metyl, October 2017. In the sections further below study summaries have been copied from the RAR (and in a few cases from the DRAR on carbendazim).

The data on thiophanate-methyl and the major metabolite carbendazim (more toxic than the parent compound, therefore relevant for the environmental classification) that was considered reliable and relevant for C&L are summarised in the table below. For completeness, data on all metabolites and transformation products observed as >5% of the applied radioactivity are included in the table.

Where half-lives were determined by SFO (single first order kinetic equation) only the DT_{50} is stated in the table (since the SFO DT_{90} is always 3.32 x DT_{50}). Where the quoted DT_{50} was derived by other kinetic models, also the DT_{90} is mentioned.

Reliable half-lives could not be determined for all metabolites/transformation products in all test systems. There may be various natural reasons for this, such as presence of metabolite only at low levels and/or only at few sampling points, varibility between replicates and therefore failure to fulfil statistical requirements, no clear degradation pattern observed over the course of the study etc. Hence, the studies as such may very well be considered reliable despite a lack of reliable half-lives for all metabolites/transformation products observed.

Some of the metabolites not identified in the original studies were later identified. Molecular structures of the main metabolites/transformation products are shown in the next table.

Table 62: Summary of relevant information on rapid degradability.

Method	Results	Remarks	Reference
OECD TG No 301 C	Study on ready biodegradability of Thiophanate-methyl. The substance was not ready biodegradable (4% biodegradation). Primary degradation was observed, probably to the largest extent caused by hydrolysis. The main metabolite was Carbendazim (18% after 28 days). Another metabolite observed above 5% was AV-1951 (7% after 28 days).	Reliable	Tsushima S. (2013) (kinetic re-evaluation elsewhere)
German guideline, conforms to US EPA TG 161-1	Hydrolysis study on Thiophanate-methyl. Results at 22°C: pH 5: Stable over 35 days. pH 7: DT_{50} 46.8 days (SFO). Transformation products observed: Carbendazim (28.6% after 33 days). Carbendazim was stable. AV-1951 (8.1% after 33 days), half-life not available. pH 9: DT_{50} 1.0 day (SFO). Transformation products observed: Carbendazim (58.7% after 4 days). Carbendazim was stable. AV-1951 (24.9% after 4 days). DT_{50} 5.4 days (SFO-SFO) but this DT_{50} was considered uncertain.	Reliable (though kinetic results for AV-1951 uncertain)	Soeda Y. & Nomura O. (1986) (kinetic re-evaluation elsewhere)

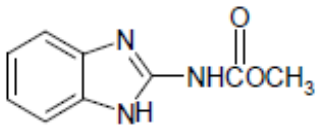
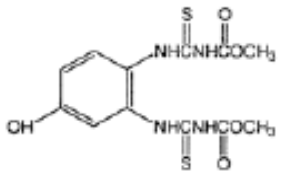
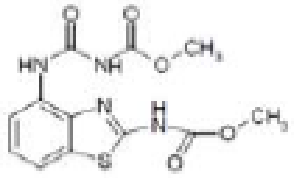
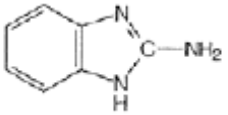
Method	Results	Remarks	Reference
OECD TG No 111	Hydrolysis study on Carbendazim. Degradation of Carbendazim was <10% after 5 days at pH 4 and 7 and 50°C. Therefore the main test was carried out at pH 9 at 45°C, 55°C and 65°C. The interpolated DT_{50} at 20°C was 435 days (SFO). Transformation product observed was 2-AB (not quantified).	Reliable	Shiotani H. (2003) (kinetic re-evaluation elsewhere)
OECD TG No 309	Study on degradation of Thiophanate-methyl in lake water (pelagic system) at 20°C, at low and high dose. pH varied 7.6-9.0 over the test. Mineralisation was ≤0.5% (by study end at 30 days). Thiophanate-methyl DT_{50} 0.64 days (low dose, DFOP, DT_{90} 5.0 days) and 2.2 days (high dose, SFO). Observed metabolites at low dose: Carbendazim, max 82.8% (14 days), DT_{50} 64.8 days (DFOP-SFO), FH-432, max 5.6% (1 day), half-life not available, UM 1, max 11.4% (30 days), half-life not reliable, UM 2, max 8.0% (30 days), half-life not available, 2-AB, max 9.5% (30 days), half-life not available. Observed metabolites at high dose: Carbendazim, max 73.4% (14 days), half-life not reliable FH-432, max 11.3% (4 days), DT_{50} 5.2 days (SFO-SFO) UM 1, max 8.7% (30 days), half-life not available, UM 2, max 5.3% (30 days), half-life not available, 2-AB, max 6.4% (30 days), half-life not available.	Reliable (but the duration of the study did not allow reliable estimation of degradation rates for all metabolites)	Hurst (2015) (kinetic re-evaluation elsewhere)
SETAC guideline and German guideline	Water/sediment study on Thiophanate-methyl, in pond and river systems, at 20°C. pH of waters 7.7-8.4, pH of sediments 7.6-7.8. Mineralisation ($^{14}CO_2$) was 1.3-1.6% (after 100 days). Thiophanate-methyl DT_{50} 3.5 days (pond system, SFO) and 1.6 days (river system, SFO). Observed metabolites in pond system (samples taken until 301 days): Carbendazim, max 75.1% (16 days), DT_{50} 76.2 days (SFO-SFO), 4-OH-TM, max 8.6% (30 days), DT_{50} 32.4 days (SFO, calculated over decline from peak), AV-1951, max 6.1% (2 days), half-life not available, 2-AB, max 7.5% (58 days), DT_{50} 189 days (SFO, calculated over decline from peak), M10, max 9.3% (140 days), half-life not reliable. Observed metabolites in river system (samples taken until 100 days): Carbendazim, max 81.6% (8 days), DT_{50} 91.6 days (SFO-SFO), 4-OH-TM, max 9.5% (8 days), DT_{50} 26.2 days (SFO, calculated over decline from peak), AV-1951, max 6.3% (2 days), half-life not available 2-AB, max 6.5% (16 days), half-life not available.	Reliable	Voelkl S. (2001) (kinetic re-evaluation elsewhere)
SETAC	Water/sediment study on Carbendazim in two systems (Bickenbach and Unter Widdersheim), at 20°C. pH of waters 8.1-8.5, pH of	Reliable	Knoch E. (2001) *,

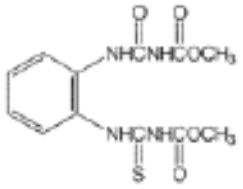
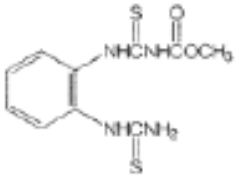
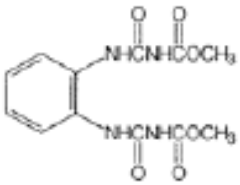
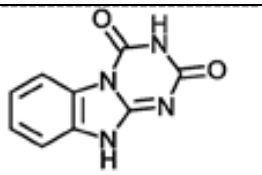
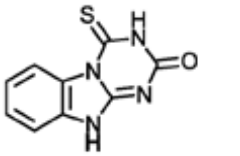
Method	Results	Remarks	Reference
Europe, Dutch guide-line	sediments 7.5-8.0. Mineralisation ($^{14}CO_2$) was 4.7-20.4% (by study end, at 120 days). Carbendazim DT_{50} 15.1 days (SFO, Bickenbach system) and 75.2 days (SFO, Unter Widdersheim system). Observed metabolites: 2-AB, max 6.3% (76 days), half-life not available.		cited in EFSA (2010) (kinetic re-evaluation elsewhere)
Draft OECD TG No 307; USA EPA TG 162-1	Study on degradation of Thiophanate-methyl in aerobic soil at 20°C, three soils with pH 5.8-7.5. Mineralisation ($^{14}CO_2$) reached 7.3-25.7% (by study end, day 120). DT_{50} 0.44-0.70 days (n=3, SFO). Metabolites observed: Carbendazim, max 62.8-75.8% (3-7 days), DT_{50} 22.0-63.2 days (n=3, SFO-SFO), CM-0237, max 4.1-9.8% (1-7 days), DT_{50} 2.8-86.5 days (n=3, SFO-SFO) 2-AB, max 2.3-6.1% (14 days), DT_{50} 11.5-13.5 (n=2, SFO-SFO).	Reliable	Voelkl S. (2002) (kinetic re-evaluation elsewhere)
OECD TG No 307	Study on degradation of Thiophanate-methyl in one aerobic soil at 20.9°C, pH 7.9. Mineralisation ($^{14}CO_2$) reached 7.6% (by study end, day 120). DT_{50} 0.27 day (SFO). Metabolites observed: Carbendazim, max 48.3% (3 days), DT_{50} 40.1 days (SFO-SFO), CM-0237, max 4.4% (28 days), half-life not available, 2-AB, max 3.8% (14 days), DT_{50} 5.3 days (SFO-SFO)	Reliable	Adam D. (2014, 2016)
BBA leaflet no. 36	Study on degradation of Carbendazim in soil at 22°C (two soils), pH 6.8 and 5.2. A complete mass balance was not provided. DT_{50} 37 and 44 days (n=2, SFO). Metabolites observed: No details provided, the RMS (DE) stated that 2-AB or other extractable residues each accounted for <5% of the applied radioactivity throughout the experiment.	Reliable, but the study was poorly described and no mass balance was provided.	Otto S. (1975) *, cited in EFSA (2010) (kinetic evaluation elsewhere)
BBA-leaflet No. 36	Study on degradation of Carbendazim in soil at 15°C, 20°C and 25°C (one aerobic soil), pH 4.7. A complete mass balance was not provided. DT_{50} 34 days (15°C), 31 days (20°C), 26 days (25°C) (n=1, SFO). Metabolites observed: No details provided, the RMS (DE) stated that 2-AB or other extractable residues each accounted for <5% of the applied radioactivity throughout the experiment.	Reliable, but the study was poorly described and no mass balance was provided.	Gilde-meister H., Jordan H.J., Remmert U. (1981) *, cited in EFSA (2010) (kinetic evaluation elsewhere)

Method	Results	Remarks	Reference
US EPA TG 161-2	Study on photochemical transformation of Thiophanate-methyl in sterile buffer (pH 5) exposed to natural sunshine outdoors in Japan (latitude 35°, longitude 139°) in mid-December at ambient temperatures (2.9–16.3°C). DT_{50} 2.3 days (SFO). Additionally, the quantum yield was determied to 3.84 x 10^{-3} and from this results DT_{50}s for water bodies under clear sky conditions at 40° latitude were estimated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter). Observed transformation products: Carbendazim, max 49.7% (by study end, 5.5 days), no half-life available, DX-105, max 14.3% (5.5 days), no half-life available. Un-identified product, max 5.6% (4.0 days), not expected to be formed in significant amounts in microbially viable systems.	Reliable	Shiotani (2003) (kinetic re-evaluation elsewhere)

* The study summaries provided below on Carbendazim are derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not re-evaluated by Sweden at this stage.

Table 63: Molecular structures, chemical names (or SMILES codes) and codes of metabolites/transformation products mentioned in the above summary table.

Carbendazim **MBC**	Methyl 1H-benzimidazol-2-ylcarbamate	
4-OH-TM	Dimethyl 4,4'-(4-hydroxy-o-phenylene)bis(3-thioallophanate)	
CM-0237	Methyl 4-[2-(methoxycarbonylamino) benzothiazolyl]allophanate	
2-AB	2-amino-1H-benzimidazole	

DX-105	Methyl 4-[2-(3-methoxycarbonyl-2-thioureido)phenyl]allophanate	
AV-1951	Methyl 4-[2-(2-thioureido)phenyl]-3-thioallophanate	
FH-432	Dimethyl 4,4'-(o-phenylene)bis(allophanate))	
M10 **UM2**	Smiles code: O=C3N=C2Nc1ccccc1N2C(=O)N3	
UM1	Smiles code: O=C3N=C2Nc1ccccc1N2C(=O)N3	

11.1.1 Ready biodegradability

Based on the available data (testing according to OECD TG No 301 C; MITI (I) (Ministry of International Trade and Industry, Japan), summarised in RAR Volume 3, B.8.2.2.1, from which the study summary has been copied below), it was concluded that thiophanate-methyl is not ready biodegradable. Primary degradation was observed, but probably to the largest extent caused by hydrolysis. The main transformation product was MBC (carbendazim). Percentage biodegradation was on average 4% in 28 days. The study was considered as reliable and can be regarded as the key study for classification on biodegradability.

Reference:	**Tsushima, S (2013)** Ready biodegradability Test of Thiophanate-methyl
Report No.:	RD-02537
Document No.:	713-003
Guideline:	OECD TG No 301 C
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal.
Material and methods:	
Test material:	Thiophanate-methyl

Lot/Batch No:	YS-0175
Purity:	99.3%
Activity control:	Aniline (purity 100%)
Activated sludge:	Standard activated sludge was obtained from the Chemicals Evaluation and Research Institute, Japan.
Test concentration:	Test material and positive control: 100 mg/L Activated sludge: 30 mg/l
Mineral medium:	Prepared from stock solutions A-D as recommended in OECD TG No 301 C
Test description:	Six bottles were prepared for the measuring of biological oxygen demand (BOD). The total volume of test solution in in each bottle was 300 ml. • Bottle No. 1 (abiotic control): water and test material. • Bottles No. 2-4 (test suspensions): mineral medium, activated sludge and test material. • Bottle No. 5 (activity control): mineral medium, activated sludge and aniline • Bottle No. 6 (inoculum blank):mineral medium and standard activated sludge To measure nitrification another six bottles prepared as described above was set up. The bottles were incubated under stirring at 25.3 - 25.8 °C for 28 days. The BOD was measured with an enclosed respirometer. The contents of the second set of bottles were filtered and the concentration of ammonium ion in the solution of each bottle was determined by ion electrometry. The pH of test systems was measured at day 0 and day 28.
Method of analysis:	HPLC and LC/MS were used to identify and quantify test material and transformation products. Reference items: MBC (carbendazim), 2-AB, AV-1951, DX-105 and 4-OH-TM

Results

Biological oxygen demand

The amount of oxygen uptake of the inoculum blank system (bottle 6) after 28 days was 2.359 mg and the amount of oxygen uptake of the abiotic control (bottle 1) was 0 mg. The amount of oxygen uptake of the test suspensions after 28 days was 4.134, 4.777 and 4.667 mg for bottle No. 2, 3 and 4, respectively corresponding to an average biodegradation of 4%. The percentage biodegradation of the activity control (aniline) was 87% after 7 days and 100% after 28 days confirming that the inoculum was viable. The results of the BOD measurements in the set of bottles used for the confirmation of nitrification were similar.

Confirmation of Nitrification

The production rate of ammonia in the test suspensions, was nearly zero indicating no biodegradation. For the activity control (aniline) the production rate of ammonia was 95.7% (average of triplicate measures).

Primary degradation

The mean residual amount of thiophanate-methyl in the test suspensions was 19.5 mg corresponding to an average disappearance rate of 35%. Three degradation products were identified and quantified with HPLC and LC/MS: Carbendazim (MBC), methyl N-[2-(thioureido)phenylaminocarbonothioyl]carbamate (AV-1951) and 2-aminobenzimidazole (2-AB). One peak in the chromatogram could not be identified but this peak was less than 1% of

the area of thiophanate-methyl. Carbendazim (MBC) was the main transformation product accounting for approximately 18% of the total recovery after 28 days while AV-1951 accounted for 7% and 2-AB for 0.3%.

The disappearance rate of thiophanate-methyl in the abiotic control was 32%. The same transformation products were identified but at a lower rate. However, the total recovery was ca 12% lower than for the test suspensions. The results indicate that hydrolysis was the main mechanism for the transformation of thiophanate-methyl.

Table 64: Thiophanate-methyl – Biological oxygen demand and % degradation after 28 days of incubation at 25°C in a ready biodegradability test according to OECD TG No 301 C.

Bottle No.	Test system	O_2 uptake day 28 (mg)	degradation (%)	Average degradation (%)
1	Abiotic control	0.000	-	-
2	Sludge + test subst.	4.134	3	4
3		4.777	4	
4		4.667	4	
5	Positive control (Aniline)	74.23	100	-
6	Inoculum blank	2.359	-	-

Table 65: Thiophanate-methyl remaining and transformation products formed after 28 days of incubation at 25°C in a ready biodegradability test according to OECD TG No 301 C.

Bottle No	Test system	Thiophanate-methyl				MBC (Carbendazim)	AV-1951	2-AB	Total recovery (%)
		Initial amount (mg)	Residual amount day 28 (mg)	Residual rate day 28 (%)	Disappea-rance rate (%)	Conversion rate day 28 (%)	Conversion rate day 28 (%)	Conversion rate day 28 (%)	
1	Abiotic control	30.02	20.53	68.4	32	6.5	3.4	0.0	78.3
2-4	Sludge + Test substance	30.02 *	19.5 *	65.1 *	35 *	17.9 *	6.9 *	0.3 *	90.1 *

* Average of 3 replicates

RMS comments and conclusion

The study seems to have been well performed and the validity criteria of the guideline were fulfilled.

It can be concluded that thiophanate-methyl is not ready biodegradable. Primary degradation was observed, probably to the largest extent caused by hydrolysis. The main transformation product was MBC (carbendazim).

11.1.2 BOD_5/COD

Not relevant, no data available.

11.1.3 Hydrolysis

The available data were summarised in RAR Volume 3, B.8.2.1.1 (study summaries copied below). The RMS concluded that thiophanate-methyl is stable to hydrolysis at ambient temperature (22°C) and pH-values (5–7) but hydrolytically degraded at higher pH. The major products resulting from hydrolysis are carbendazim (MBC) and AV-1951 at pH conditions in which hydrolysis occurred. Carbendazim is considered as stable under environmentally relevant conditions (pH, temperature).

Reference:	1. **Soeda, Y. and Nomura, O. (1986)** Thiophanate-methyl – Hydrolysis. 2. **Kiesel, A. and Geibel, E. (2015c)** Assessment of degradation kinetics of thiophanate-methyl in a hydrolysis study according to the recommendations of the FOCUS report on degradation kinetics (2006, 2014). 3. **Kiesel, A. and Geibel, E. (2015d)** Raw data to the assessment of degradation kinetics for thiophanate-methyl based on data of a laboratory hydrolysis study according to FOCUS degradation kinetics (2006, 2014) related to the supplementary dossier - M-CA Section 7.
Report No.:	1. EC-67 (RD-8679) 2. RD-03328 3. RD-03370
Document No.:	1. 711-001 2. 782-037 3. 782-043
Guideline:	1. BBA Merkblatt No. 55 Part 1 (also conforms to later US EPA Guideline 161-1) 2., 3. FOCUS degradation kinetics (2006, 2014)
GLP:	1. No 2. and 3. not applicable
Previous evaluation:	1. In DAR (1997) 2. and 3. submitted for the purpose of renewal.
Material and methods:	
Test material:	Thiophanate-methyl, unlabelled
Lot Batch No:	GP-73
Purity:	99.6%
Test concentration:	10 mg/L thiophanate-methyl
Test system and conditions:	Stock solution of 250 mg thiophanate-methyl in 100 ml acetone was prepared and diluted in buffers (2 ml stock solution in 500 ml buffer). 30 mL buffered test solution in each 50 mL stoppered sterilised Erlenmeyer flask was monitored in a dark incubator. Experimental temperatures were 22, 45 and 65°C. Buffers: Clark-Lubs solutions (potassium hydrogen phthalate and sodium hydroxide; pH 5), Sorensen solution (potassium dihydrogen phosphate; pH 7), and Clark-Lubs (boric acid + potassium chloride and sodium hydroxide; pH 9). De-ionised water was used and all buffers were filtered through 0.2 µm membrane filter for sterilisation. Tests were conducted during 35 days. The test was performed with duplicate samples for each temperature and for each pH-value.
Method of analysis:	HPLC (UV detector-280 nm), thin-layer chromatography, and mass spectroscopy (EI-DI) were used to identify products formed. A first preliminary test was carried out at 65°C for a short period of time to identify major hydrolysis products. The second part of the study quantified the relative amounts of parent and hydrolysis products formed over time at different pH and temperatures as above. Quantification was done by comparison of HPLC-MS diagrams with standard solutions of available reference substances.
Used standards:	The following reference standards were used: Carbendazim (MBC), DX-105, AV-1951, FH-432 and 2-AB.
Kinetic evaluation:	In the original report, hydrolysis rate constants and half-lives were calculated with SFO equation, and interpolated with Arrhenius equation for temperature adjustment to 25°C. Degradation rates were determined later by Kiesel and Geibel (2015c,d), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.

Results

No contamination of incubation samples by bacteria/fungi was observed over the experiment, and the pH remained stable throughout the experiment. In the study report results over all sampling days were presented as mg/l parent equivalents. The RMS has expressed this as % AR (parent equivalents) for the last sampling interval (see table below). Hydrolysis occurred over the pH range 5-9 and was most rapid for the parent substance at the highest pH. The hydrolysis rate also substantially increased with temperature (22–65°C).

Table 66: Recovery in % of initial amount of thiophanate-methyl for the parent and its hydrolysis products over the course of the experiment in Soeda and Nomura (1986), calculated by the RMS.

pH, temperature and final sampling time	thiophanate-methyl	MBC	DX-105	AV-1951	2-AB	Total
pH 5, 22°C, 35 d	98.1	0	0	0	0	98.1
pH 5, 45°C, 35 d	61.3	29.2	0	2.7	0	93.2
pH 5, 65°C, 168 h	14.6	68.2	0	2.4	12.4	97.6
pH 7, 22°C, 33 d	59.6	28.6	0	8.1	0	96.3
pH 7, 45°C, 137 h	16.7	67.2	0	11.2	0	95.1
pH 7, 65°C, 3.5 h	21.8	66.7	0	8.1	0	96.6
pH 9, 22°C, 96 h	6.5	58.7	0	24.9	0	90.1
pH 9, 45°C, 2 h	21.2	59.8	0	15.8	0	98.8
pH 9, 65°C, 8 minutes	22.5	61.5	0	11.4	0	95.4

Hydrolysis products were identified as carbendazim (MBC), AV-1951 and 2-AB. At 22°C MBC was found as maximum 58.7% (pH 9, last sampling point) and AV-1951 was found as max 26.4% (pH 9, sampling point at 75 hours). 2-AB was only found in the experiment at pH 5 and 65°C, at concentration that increased over the incubation period, to 12.4%. No other compounds were found in the test solutions in the definitive test.

In the original report half-lives of thiophanate-methyl (at 25°C) were estimated to 867 days (pH 5), 36 days (pH 7) and 0.7 days (pH 9). These results are superseded by the kinetic re-analyses in Kiesel & Geibel (2015c,d). Data from the experiments at 22°C, pH 7 and 9 were included in the analyses (in mg/l parent equivalents). Data from both replicates were used. For the experiment at pH 7 thiophanate-methyl was assumed to degrade into MBC in a second step of a pathway fit. For the experiment at pH 9 thiophanate-methyl was assumed to degrade into MBC and AV-1951 in step 2, and in a 3rd step formation of MBC from AV-1951 was added.

SFO was selected as the best fit model for the parent (for data from experiment at pH 7 the Chi2 error-% was lowest in SFO, and for the experiment at pH 9 the confidence interval of both α and β included zero). The SFO (and SFO-SFO) results are summarised in the tables below. Visual assessments are not shown but were good in all cases. Carbendazim increased over the duration of the test and no reliable parameters could be determined as demonstrated by the p-value 0.5.

Table 67: Summary of kinetic re-analysis by Kiesel and Geibel (2015c,d) of the hydrolysis data from Soeda and Nomura (1986).

Substance	Test condition	Kinetic model	Mo	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Parent only fit										
Thiophanate-methyl	pH 7 22°	SFO	9.93	k=0.01481	1.4	$2.4x10^{-10}$	0.01	0.02	46.8	155.5
	pH 9 22°	**SFO**	9.97	k=0.6881	0.6	$1.5x10^{-15}$	0.7		1.0	3.3
Pathway fits										
Carbendazim	pH 7 22°	SFO-SFO	n.r.	$k=2.3x10^{-14}$	2.8	0.5	-0.01	0.01	>1000	>1000
	pH 9 22°	SFO-SFO	n.r.	$k=2.2x10^{-14}$	0.9	0.5	-0.2	0.2	>1000	>1000
AV-1951	pH 9 22°	SFO-SFO	n.r.	k=0.1273	0.8	$2x10^{-16}$	0.11	0.13	5.4	18.1

n.r. Not relevant.

Table 68: Formation fractions of carbendazim and AV-1951, from kinetic re-analyses by Kiesel and Geibel (2015c,d) of the data from Soeda and Nomura (1986).

Formation	Test condition	Formation fraction	St. dev.
Thiophanate-methyl → Carbendazim	pH 7 22°	0.69	0.08
	pH 9 22°	0.62	0.02
Thiophanate-methyl → AV-1951	pH 9 22°	0.38	0.005
AV-1951 → Carbendazim	pH 9 22°	0.057	1.6 [a]

a RMS comment: The large standard deviation demonstrate that the formation fraction was unreliable.

RMS comments and conclusion

The study was considered acceptable in the DAR (1997) and is considered acceptable also for the purpose of renewal. No mass balance was reported (non-radiolabelled test substance use) but the total recovery was always ≥9 mg/l parent equivalents.

The analytical techniques were acceptable for the identification of parent and transformation products although the method for quantification (HPLC peak height) was relatively un-precise. Test concentrations were above detection limits and limits of quantification for all reference compounds but 2-AB. This was a non-GLP study but the experiments were scientifically sound. It is not considered likely that repetition of the study would alter the conclusion substantially.

The data showed that thiophanate-methyl is stable to hydrolysis at ambient temperature (22°C) and pH-values (5–7). The RMS concludes that the major products resulting from hydrolysis are carbendazim (MBC) and AV-1951 at pH conditions in which hydrolysis occurred.

The kinetic re-analyses are accepted by the RMS. However, for the experiment at pH 9 Kiesel & Geibel (2015c,d) mixed up hours and days and the results were erroneously presented in hours instead of days (e.g., DT_{50} for parent was given as 1.0 hour but should have been 1.0 day, for AV 1951 DT_{50} was given as 0.23 days but should be 5.4 days). For AV-1951 statistically and visually acceptable results were obtained however the RMS notes that the DT_{50} of 5.4 days was extrapolated beyond the study duration of 4 days (96 hours) and therefore uncertain. Additionally, it is questionable if results can be accepted when the parameters for the other product (MBC) simultaneously optimised were unreliable.

Reference:	1. **Shiotani, H. (2003b)** Hydrolysis Study of MBC (ID No. 2-9-13) 2. **Kiesel, A., Geibel, E. (2015e)** Assessment of degradation kinetics of MBC in a hydrolysis study according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014) 3. **Kiesel, A., Geibel, E. (2015f)** Raw data to the assessment of degradation kinetics for MBC based on data of a laboratory hydrolysis study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7
Report No.:	1. RD-02326 2. RD-03327 3. RD-03371
Document No.:	1. 711-002 2. 782-038 3. 782-044
Guideline:	1. OECD TG No. 111 (1981) 2. and 3. FOCUS degradation kinetics (2006, 2014)
GLP:	1. Yes (RMS comment: not signed) 2. and 3. not applicable
Previous evaluation:	All three studies submitted for the purpose of renewal.
Material and methods:	
Test material:	Carbendazim (Methyl benzimidazol-2-ylcarbamate, MBC) unlabelled
Lot/Batch No.:	31-8214-C
Purity:	99.8%, HPLC determined
Test concentration:	3.0 mg/L.
Test system and conditions:	Stock solutions of ca 3.0 mg MBC in 10 ml methanol:acetonitrile (2:8, v/v) were prepared and 2.0 ml diluted in 200 ml buffer. Buffers: pH 4.0 (AcOH and AcONa), pH 7.0 (NaOH and KH_2PO_4), pH 9.0 (NaOH and H_3BO_3). The initial test to determine stability was done at 50°C and pH 4, 7 and 9 during 5 days in the dark. The main test was done only at pH 9 during 0–21.8 days at 45°C, 0–4.96 days at 55°C, and during 0–2.04 days at 65°C tests, in the dark. All test solutions were sterile. (The intention was to carry out the main test at 25 and 35°C but the OECD TG requirement of 6 data points in the range 20–70% degradation was not met and therefore higher temperatures were finally used.)
Method of analysis:	HPLC (UV detector 282 nm) and mass spectroscopy (EI-DI) were used to identify products formed. The test was performed with duplicate samples for each temperature and for each pH-value. The main study quantified the relative amounts of MBC and hydrolysis products formed over time at pH 9 and temperatures as above. Quantification was done by comparison of HPLC-MS diagrams with standard solutions of available reference substances.
Used standards:	MBC and 2-AB (2-Aminobenzimidazole).
Kinetic evaluation:	Hydrolysis constants and half-lives were calculated with SFO equation and interpolated with Arrhenius equation for temperature adjustment to 25°C in the study report. Degradation rates were re-assessed by Kiesel and Geibel (2015e,f), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.

Results

Sterile conditions were maintained and pH remained stable throughout the experiment.

Degradation of carbendazim (MBC) at pH 4 and 7 was less than 10% in all samples after 5 days at 50°C, but exceeded 10% in pH 9 samples after 5 days. Therefore, the main test was conducted only at pH 9.

Carbendazim decreased from the initial concentration of 3.0 mg/l to 0.85-1.05 mg/l after 22 days at 45°C, after 5 days at 55°C and after 2 days at 65°C. HPLC chromatograms provided showed only presence of carbendazim and 2-AB in all solutions. The amounts of 2-AB were not quantified.

The estimated hydrolysis DT_{50} at pH 9 and 25°C was calculated in the study report to 200 days.

The results from Shiotani (2003b) were superseded by the kinetic re-analyses by Kiesel and Geibel (2015e,f). Data from the experiments at pH 9 and 45, 55 and 65°C were included in the analyses (in mg/l). Data from both replicates were used. For the results at 45 and 55°C the confidence intervals of α and β included zero and therefore the SFO results were more reliable than the FOMC results (Chi2 error-% was also marginally lower in SFO). At 65°C both kinetic models provided acceptable and very similar results, and the results from SFO was selected as endpoints. All visual assessments were good. The results are summarised in the table below.

As the tests were conducted only at 45, 55 and 65°C and as these temperatures do not represent realistic environmental conditions, the DT_{50} and DT_{90} values derived by SFO for MBC were extrapolated to 20°C by the Arrhenius equation. The interpolated (20°C) DT_{50} value was 435 days and the corresponding DT_{90} 1444 days.

Table 69: Summary of kinetic re-analysis by Kiesel and Geibel (2015e,f) of the hydrolysis data from Shiotani (2003b).

Substance	Test condition	Kinetic model	Mo	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Carbendazim	pH 9 45°	SFO	3.02	k=0.05484	1.2	$<2x10^{-16}$	0.05	0.06	12.6	42.0
	pH 9 55°	SFO	2.98	k=0.2019	1.4	$<2x10^{-16}$	0.19	0.21	3.4	11.4
	pH 9 65°	SFO	3.02	k=0.6345	1.1	$<2x10^{-16}$	0.62	0.65	1.1	3.6
		FOMC	3.04	α=5.815 β=2.194	0.85	n.r.	1.5 1.7	10.1 15.2	1.1	4.1

n.r. Not relevant.

RMS comments and conclusion

The study is considered acceptable. The OECD guideline was followed and the presented data is acceptable for the purpose of determining the half-life for hydrolysis of MBC. Amounts of 2-AB were not quantified but since the precursor carbendazim can be considered as hydrolytically stable at environmentally realistic pH values and temperatures this is not considered as a deficiency of the study.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Field dissipation and monitoring data were available but not considered relevant for C&L.

Just for information: The field dissipation DT_{50}s for thiophanate-methyl were 0.99-1.8 days in two Italian soils (pH 7.7-8.0) and 1.8-3.3 days in two German soils (pH 5.8-6.2) (all SFO). For the same trials field dissipation DT_{50}s for carbendazim were estimated to 13.6-18.9 days (Italian sites) and to 22.1-24.8 days (German sites) (all SFO-SFO). When the data from these field trials were normalised to 20°C and field

capacity moisture conditions, the DT_{50}s for thiophanate-methyl were 1.1-6.5 days (Italian sites) and 0.8-1.7 days (German sites), and the DT_{50}s for carbendazim were 23.1-33.3 days (Italian sites) and 13.6-19.2 days (German sites).

11.1.4.2 Inherent and enhanced ready biodegradability tests

No relevant data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Individual studies were summarised in the RAR Volume 3 B.8.2.2 (water and water/sediment) and B.8.1.1 (soil), respectively, and the below study summaries have been copied from the RAR.

In the study on mineralisation in surface water (Hurst, 2015) as well as in the water/sediment study (Voelkl, 2001) carbendazim was formed as >80% of the applied radioactivity. Thiophanate-methyl had a relatively short half-life in the water-only system (DT_{50} 0.64-2.2 days) as well as in the water/sediment systems (DT_{50} 1.6-3.5 days), whereas degradation of carbendazim was considerably more slow (DT_{50} 64.6 days in the water-only system and 76.2-91.6 days in water/sediment systems). Additional DT_{50}s of 15.1-75.2 days in water/sediment systems were presented in study with carbendazim as test substance.

The same conclusion could be drawn from studies on degradation in soil; carbendazim was the major metabolite formed (max 75.8%), and while the half-lives for thiophanate-methyl were short (< 1 day) the half-lives for carbendazim were considerably longer (22-63.2 days). Additional DT_{50} presented in studies with carbendazim as test substance were in the same range.

Reference:	1. **Hurst, L. (2015)** Thiophanate-methyl: Aerobic Mineralisation of ^{14}C-Thiophanate-methyl in Surface Water 2. **Geibel, E. and Lobe, I. (2015)** Assessment of degradation kinetics of Thiophanate-methyl in pelagic water under aerobic laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014) 3. **Geibel, E. (2015**) Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a pelagic water study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7 4. **Geibel, E. and Lobe, I. (2016)** Assessment of degradation kinetics of Thiophanate-methyl in pelagic water under aerobic laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014) **5. Geibel, E. and Moshenberg, K. (2016)** Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a pelagic water study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7
Report No.:	1. RD-03335 2. RD-03402 3. RD-03403 4. RD-03402N 5. RD-03403N
Document No.:	1. 714-002 2. 782-036 3. 782-042 4. 782-090 5. 782-091

Guideline:	1. OECD TG No 309 2. - 5. FOCUS Degradation Kinetics report (2006, 2014)
GLP:	1. Yes 2. - 5. Not applicable
Previous evaluation:	Submitted for the purpose of renewal.

Material and methods:

Test material:	[Ring-U-^{14}C] labelled -Thiophanate-methyl
Lot/Batch No:	XX/41/2
Radiochemical Purity:	98.46% (as quoted on the Certificate of Analysis), 95.7% within the application solution, as determined in the study
Test concentration:	Low dose: 10 µg/l High dose: 95 µg/l The stock solution was prepared in acetone. Concentration of acetone in final test solutions was <1%.
Test system:	Route and rate of degradation of thiophanate-methyl was studied in natural water from a lake (pelagic system) at a high (95 µg/l) and a low concentration (10 µg/l). The test system consisted of glass flasks, connected to two 2M NaOH, traps to capture CO_2. Each flask was aerated with moistened air. Air passing through sterilised samples was passed through bacterial air filters positioned at the inlet and outlet of each flask to maintain sterility. Samples (100 mL) of natural water (100 um sieved) were used in the tests. The water used had a suspended solids concentration of 2.94 mg/L. Thiophanate-methyl dissolved in acetone was applied onto the water surface using a syringe.

There were seven incubation groups for the main test:

Group	Condition *	No. of flasks
A: Low test conc.	Thiophanate-methyl (10 µg/l) + acetone (712 mg/l)	18 **
B: High test conc.	Thiophanate-methyl (95 µg/l) + acetone (759 mg/l)	18
C: Abiotic control (sterilised)	Thiophanate-methyl (95 µg/l) + acetone (759 mg/l)	2
D: Activity control	Sodium [^{14}C]benzoate (10 µg/l)	2
E1:Blank control	Acetone (759 mg/l)	1
E2: Blank control	Untreated	1
F: Activity/solvent control	Sodium [^{14}C]benzoate (10 µg/l) + acetone (759 mg/l)	2

** RMS comment: In Hurst (2015) the concentration of acetone was apparently given in an erroneous unit (µg/l). The correct unit should be mg/l, as stated above.*
Example: Blank control E1:
96 µL acetone = 0.096 mL acetone
0.096 mL acetone x density 0.791 g/mL = 0.0759 g acetone
which was added to test solution of 100 mL:
0.0759 g acetone / 0.1 L water = 0.759 g/L = 759 mg/L.
***14 + 4 spares*

Test conditions:	Samples were incubated under dark conditions at 20 ± 2°C under continuous stirring.
Sampling time points:	High and low dose: Day 0, 1, 2, 4, 7, 14 and 30 (duplicate samples). Sterile control: Day 0 and 2 (duplicate samples). Activity control: Day 2, 7, 14 and 30 (single samples). CO_2-traps were assayed whenever the associated samples were removed for analysis. Traps associated with the activity controls were assayed at days 0, 3, 7, 14 and 30. Oxygen and pH in water were measured in the blank controls at each sampling interval.
Method of analysis:	At the appropriate sampling interval, water was aspirated from the flask and

	radioactivity was quantified by LSC. Acetone (25 mL) was used to rinse the vessel to ensure all radioactivity had been transferred. No organic wash sample contained >3% AR thus none were analysed chromatographically. The water phase was analysed by HPLC. Confirmation of degradation products in selected samples was done by TLC and structural elucidation of two unknown metabolites was performed by LC-MS of a single sample. Reference items: FH-432, DX-105, Carbendazim (MBC), 2-AB, CM-0237, CM-0238, 4-OH-TM and AV-1951.
Kinetic analysis:	Degradation rates were determined in the study. These results were superseded by kinetic re-evaluations performed in accordance with FOCUS (2006, 2014) and using KinGUI 2.1 software. The first kinetic re-evaluation (Giebel & Lobe, 2015, and Geibel, 2015) was not accepted by the RMS since erroneous input data were used for the optimisations and since the KinGUI results were not presented in Word or pdf format. During the evaluation the applicant therefore submitted a revised kinetic re-evaluation (Giebel & Lobe, 2015, and Geibel & Moshenberg, 2016). The results presented below are all from the revised kinetic re-evaluation.

Table 70: Water characteristics.

		Lake water
Source		Fountains Abbey, Studley Royal, Ripon, UK The lake is surrounded by parkland and no pesticides had been used in the vicinity 10 years prior to sampling
Storage and handling		The water was collected into plastic containers via a 100 µm sieve
Temperature	[°C]	6.4 (at sampling) Prior to use the water was stored in the dark 4 ± 2°C, with free access to air.
pH		8.73
Conductivity	[µS/cm]	236
Redox potential	[mV]	189.2
Oxygen content	[mg/l]	11.83
Colour		Clear, slightly yellow
Alkalinity	[mg $CaCO_3$/l]	104
Hardness	[mg $CaCO_3$/l]	146
Total organic carbon (TOC)	[mg C/l]	4.55
Dissolved organic carbon (DOC)	[mg C/l]	4.51
Total Phosphorous	[mg/l]	0.19
Total nitrogen	[%]	0.00938
Total nitrate	[mg/l]	1.7
Total nitrite	[mg/l]	0.021
Ammonium (NH_4+)	[mg/l]	0.152
Suspended solids	[mg/l]	2.94
BOD_5	[mgO_2/l]	11.99

Results

The pH during the test was between 7.6 and 9.0 and the water was maintained under aerobic conditions (oxygen concentrations 6.1 to 9.4 mg/L). Comparison of the activity control treated with acetone and that not treated with acetone showed similar results. In both units, >80% of the applied sodium benzoate had mineralised within 14 days, showing that the water was microbially active with or without the addition of acetone. Comparison of the blank control unit treated with acetone and that not treated with acetone showed similar water quality results.

There was almost no mineralisation (≤ 0.5% AR) and most of the radioactivity remained in the water. Only small amounts of AR were detected in the organic vessel wash. Total recovery of radioactivity (mass balance) was ≥ 94% AR in all units sampled. An overview of the distribution at start and end of the test is given in the table below.

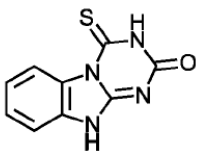
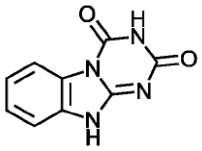
Table 71: Material balance in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) over 30 days of incubation. As % of applied radioactivity, mean of duplicates (single samples for high dose, sterile system).

Incubation group	Treatment rate (µg/l)	Time (DAT)	Recovery of radioactivity (% AR)			
			Aqueous	Organic wash	Traps	Mass balance
A (low dose)	10	0	97.7	1.0	NA	98.7
		30	96.4	1.4	0.5	98.3
B (high dose)	95	0	94.2	1.0	NA	95.1
		30	93.2	1.5	0.5	95.1
C (high dose, sterile)	95	0	98.3	0.6	NA	98.9
		2	99.3	0.9	ND	100.2

NA Not applicable/Not analysed.

ND Not detected

Thiophanate-methyl degraded to several known metabolites (including MBC, FH-432, 2-AB, CM-0237 and DX-105) and two unknown metabolites (UM1 and UM2). Following LC-MS analysis structures were postulated for the two unknown metabolites, as shown below:

Name as referenced in the study	UM1	UM2
Molecular formula	C9H7ON4S	C9H7O2N4
IUPAC name	4-Thioxo-1,3,4a,9-tetraza-1,3,9,9atetrahydrofluoren-2-one	1,3,4a,9-Tetraza-1,3,9,9atetrahydrofluorene-2,4-dione
Postulated structure		

The levels of thiophanate-methyl rapidly decreased, and by day 14 none remained. The main metabolite was carbendazim (MBC) which accounted for around 70% of the AR already after 4 -7 days of incubation depending on test concentration (see below table). Metabolite FH-432 appeared early (day 0 in the low and day 1 in the high test concentration) and reached ca 11% AR by day 14 in the high test concentration. It was however not detected at the end of incubation. UM 1 was detected at all sampling occasions in the lower test concentration and seemed to increase throughout the study, to 11.4% at the final day. At the higher test concentration UM1 appeared on day 4 and increased from 2.5% AR to 8.7% AR. The sterile units showed a similar profile, with thiophanate-methyl degrading rapidly to carbendazim (MBC) from day 0 and to FH-432 by day 2.

Table 72: Thiophanate-methyl and major metabolites in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) and over 30 days of incubation. As % of applied radioactivity, individual replicates and mean of duplicates (single samples for high dose, sterile system).

Day	Thiophanate-methyl			MBC			FH-432			UM1		
	Replicate		Mean	Replicate		Mean	Replicate		Mean	Replicate		Mean
	1	2		1	2		1	2		1	2	
Low dose 10 µg/l												
0	65.3	56.8	**61.1**	30.1	32.5	**31.3**	ND	5.9	**2.9**	ND	1.4	**0.7**
1	32.1	39.7	**35.9**	46.7	44.7	**45.7**	4.6	6.7	**5.6**	6.8	4.6	**5.7**
2	21.6	22.5	**22.1**	60.9	59.6	**60.2**	4.6	3.1	**3.9**	7.6	9.1	**8.3**
4	11.5	11.2	**11.4**	69.1	77.1	**73.1**	4.5	ND	**2.3**	7.8	8.9	**8.3**
7	6.7	7.2	**6.9**	79.9	76.3	**78.1**	ND	ND	**ND**	10.5	8.0	**9.2**
14	ND	ND	**ND**	86.2	79.4	**82.8**	ND	ND	**ND**	4.8	7.3	**6.0**
30	ND	ND	**ND**	71.2	47.5	**59.3**	ND	ND	**ND**	7.6	15.1	**11.4**
High dose 95 µg/l												
0	89.8	88.4	**89.1**	3.2	1.4	**2.3**	ND	ND	**ND**	ND	ND	**ND**
1	72.9	60.0	**66.4**	16.5	28.7	**22.6**	5.8	7.6	**6.7**	ND	ND	**ND**
2	44.8	48.7	**46.8**	40.4	37.6	**39.0**	11.2	10.8	**11.0**	ND	ND	**ND**
4	32.3	23.8	**28.0**	49.9	61.7	**55.8**	11.3	11.2	**11.3**	5.1	ND	**2.5**
7	8.5	13.1	**10.8**	71.6	65.7	**68.7**	11.5	10.6	**11.1**	5.8	6.3	**6.0**
14	ND	ND	**ND**	76.2	70.6	**73.4**	4.8	6.4	**5.6**	9.4	4.9	**7.2**
30	ND	ND	**ND**	72.3	71.8	**72.0**	ND	ND	**ND**	9.3	8.1	**8.7**
High dose 95 µg/l, sterile *												
0	**92.1**			**4.2**			**ND**			**ND**		
2	**67.1**			**23.4**			**6.9**			**ND**		

ND Not detected

* Single samples

In addition to the metabolites listed in the table above, the following metabolites were detected: DX-105, 2-AB, CM-0237 and UM2 (see below table). UM2 was detected at levels of 8.0% AR and 5.3% AR at study end in the low and high dose, respectively. 2-AB was detected at the two last sampling in both test concentrations, at max 9.5 and 6.4% AR. DX-105 and CM-0237 were considered as transient productrs as they were only detected in low concentrations at single occasions. Also other metabolites were detected (in total 6.9% AR by study end in the lower dose), but they were not further characterised. The potential transformation products 4-OH-TM, AV-1951 and CM-0238 were not detected. A tentative degradation scheme is given in the figure below.

Table 73: Additional metabolites in lake water treated with thiophanate-methyl at 10 µg/l (low dose) and 95 µg/l (high dose) over 30 days of incubation. Sampling days with only non-detects are not included. As % of applied radioactivity, mean of duplicates.

Day	DX-105	2-AB	CM-0237	UM2	Other
Low dose 10 µg/l					
1	**1.5**	ND	ND	ND	**1.5**
7	ND	ND	**2.4**	ND	ND
14	ND	**8.0**	ND	ND	ND
30	ND	**9.5**	ND	**8.0**	**6.9**
High dose 95 µg/l					
0	**1.2**	ND	ND	ND	ND
14	ND	**5.9**	ND	ND	**2.7**
30	ND	**6.4**	ND	**5.3**	ND

ND Not detected

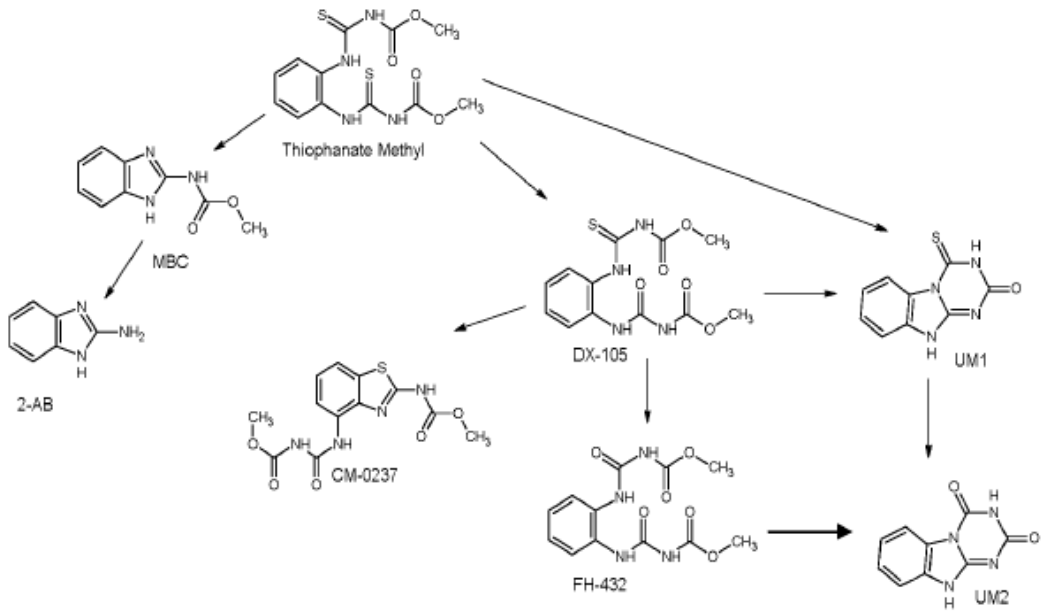


Figure 8.2.2.2-1. Proposed pathway for degradation in lake water (from Hurst, 2015).

A step-wise assessment of the degradation kinetics was conducted in accordance with FOCUS degradation kinetics guidance (Geibel & Lobe, 2016, and Geibel & Moshenberg, 2016). For the low dose test system thiophanate-methyl and degradation products carbendazim (MBC) and UM1 were considered; for the high dose test system thiophanate-methyl, carbendazim (MBC) and FH-432. FH-432 was not considered for the low dose system and UM1 not for the high dose system due to too few data points. Conceptually, all three metabolites were treated as primary metabolites. 2-AB and UM2 could not be included in the analyses as they were only detected occasionally. Data from both replicates were used and time zero amounts of metabolites were added to the parent compartment. The results are presented in tables and figures below (graphs not shown for statistically not reliable results).

At the low concentration (10 µg/) FOMC provided a lower Chi2 error-% than SFO and additional biphasic models were therefore used. DFOP was selected as the best fit model for thiophanate-methyl. At the high test concentration (95 µg/l), the SFO model provided a good fit to the data for the parent whereas FOMC parameters were not reliable.

For the metabolites acceptable results were obtained for carbendazim (MBC) in the low dose system and for FH-432 (high dose system). The authors apparently accepted also the results for UM1 (low dose) and carbendazim (MBC) (high dose) but since the rate constants did not pass the t-test the RMS did not consider these results reliable.

Table 74: Kinetic evaluation for thiophanate-methyl; lake water treated at low (10 µg/l) dose. Results for thiophanate-methyl are from parent only fit. From Geibel & Lobe (2016) and Geibel & Moshenberg (2016) based on data from Hurst (2015).

Substance	Dose rate	kinetic model	Mo	χ2, % error	parameter	Lower Confidence interval	prob > t	DT_{50} (days)	DT_{90} (days)
Thiophanate-methyl	Low	SFO	94.29	14.0	k=0.8096	0.66	5.4×10^{-7}	0.86	2.8
		FOMC	95.95	3.0	α= 1.223 ß= 0.829	0.87 0.42	n.r.	0.63	4.6
		DFOP	95.99	2.1	k1= 1.7342 k2= 0.2332 g= 0.6763	1.1 0.13 0.53	4.2×10^{-4} 1.4×10^{-3} 8.4×10^{-6}	0.64	5.0
		HS	96.00	2.7	k1= 0.9836 k2= 0.2663 tb= 1.34	0.89 0.18 1.0	1.1×10^{-8} 1.5×10^{-4} 7.3×10^{-6}	0.70	5.0
MBC	Low	DFOP-SFO	n.r.	6.4	k= 0.01070	0.0034	0.0035	64.8	215
UM1	Low	DFOP-SFO	n.r.	15.8	$k= 2.33 \times 10^{-14}$	-0.017	0.50	>1000	>1000

n.r. Not relevant

Table 75: Kinetic evaluation for thiophanate-methyl; lake water treated at high (95 µg/l) dose. Results for thiophanate-methyl are from parent only fit. From Geibel & Lobe (2016) and Geibel & Moshenberg (2016) based on data from Hurst (2015).

Substance	Dose rate	kinetic model	Mo	χ2, % error	parameter	Lower Confidence interval	prob > t	DT_{50} (days)	DT_{90} (days)
Thiophanate-methyl	High	SFO	91.66	2.5	k=0.3142	0.28	1.0×10^{-8}	2.2	7.3
		FOMC	92.28	2.4	α= 10.65 ß= 31.86	-26.6 -86.5	n.r.	- [a]	- [a]
MBC	High	SFO-SFO	n.r.	1.2	$k= 6.419 \times 10^{-4}$	-0.0038	0.39	>1000	>1000
FH-432	High	SFO-SFO	n.r.	7.1	k= 0.1334	0.10	3.6×10^{-11}	5.2	17.3

n.r. Not relevant

a Not considered acceptable.

Table 76: Formation fractions of carbendazim (MBC), FH-432 and UM1, according to kinetic re-analyses by Geibel & Lobe (2016) and Geibel & Moshenberg (2016) of the data of from Hurst (2015).

Degradation pathway	Dose rate	Formation fraction	Standard deviation
Thiophanate-methyl → MBC	Low	0.901	0.045
	High	0.768 [a]	0.044
Thiophanate-methyl → UM1	Low	0.099 [a]	0.070
Thiophanate-methyl → FH-432	High	0.232	0.074

a Result not considered reliable by the RMS since the corresponding rate constant was not reliable (p-values > 0.10).

Measured & predicted vs. Time	Residual Plot
Thiophanate-methyl, Low dose, SFO	
Thiophanate-methyl, Low dose, DFOP	
Thiophanate-methyl, High dose, SFO	

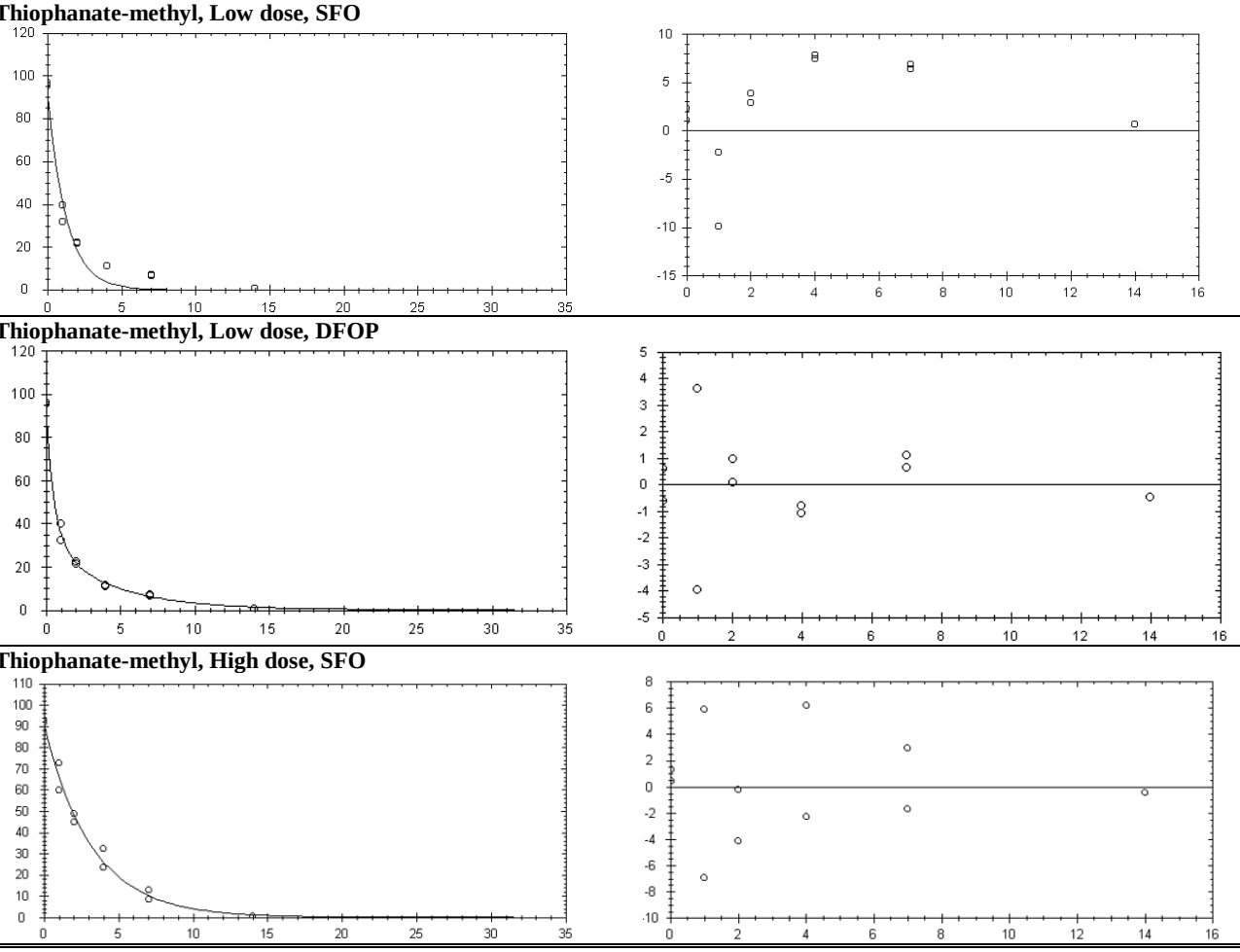


Figure 8.2.2.2-2 Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl from a surface water simulation study. Low dose (10 µg/l) and high dose (95 µg/l) (from Geibel & Moshenberg, 2016).

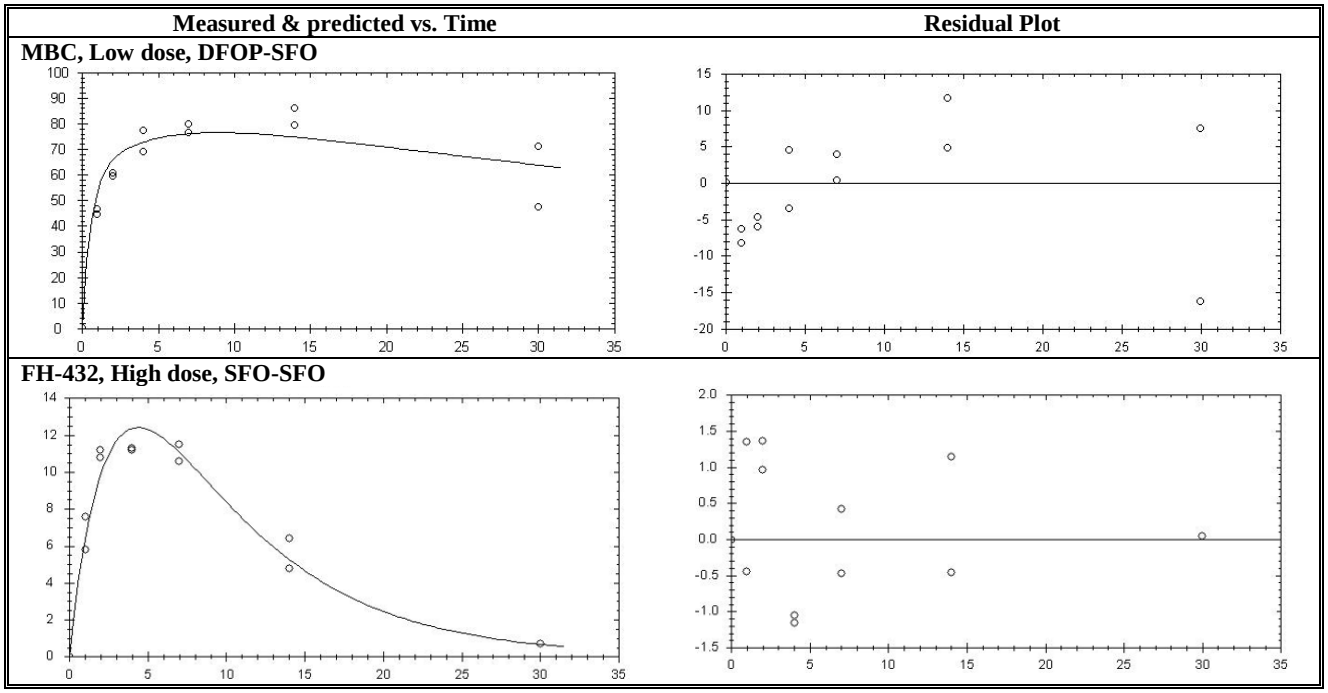


Figure 8.2.2.2-3 Visual fit and plot of residuals for kinetic fitting of data for metabolites of thiophanate-methyl in a surface water simulation study (from Geibel & Moshenberg, 2016). Only results accepted by the RMS.

RMS comments and conclusion

The observed degradation of the reference compound sodium benzoate demonstrated that the validity criteria for the test was fulfilled. The simulation study is therefore considered acceptable. However, a prolongation of the test would have enabled a more reliable estimation of the degradability of the observed transformation products.

For UM1 (low dose) and carbendazim (high dose) the rate constant did not pass the t-test and the results should not be further used.

Reference:	1. **Völkl, S. (2001)** ^{14}C-Thiophanate-methyl: Route and rate of degradation in aerobic aquatic systems 2. **Kiesel, A. and Geibel, E. (2015i)** Assessment of degradation kinetics of Thiophanate-methyl in water/sediment systems under laboratory conditions according to recommendations of the FOCUS Report on Degradation kinetics (2006, 2014) 3. **Kiesel, A. and Geibel, E. (2015j)** Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of a laboratory water/sediment study according to FOCUS Degradation Kinetics (2006, 2014) related to the supplementary dossier – M-CA Section 7 4. **Moshenberg, K. and Drechsler, S. (2017a)** Assessment of degradation kinetics of 4-OH-TM and 2-AB in water/sediment systems under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014) 5. **Moshenberg, K. and Drechsler, S. (2017b)** Raw data to the assessment of degradation kinetics of 4-OH-TM and 2-AB based on data of a laboratory water/sediment study according to FOCUS Degradation Kinetics (2006, 2014)
Report No.:	1. 735063 2. RD-03329 3. RD-03373

	4. RD-10140 5. RD-10141
Document No.:	1. 714-001 2. 782-040 3. 782-046 4. 782-092 5. 782-093
Guideline:	1. SETAC guideline (1995). BBA Guideline Part IV:5-1 (1990) 2. and 3. FOCUS Degradation kinetics report (2006, 2014)
GLP:	1. Yes 2. – 5. Not applicable
Previous evaluation:	1. In Addendum (2003) to the DAR (1997). The test description has been copied from the addendum (A brief description of the method of analysis has been added by the RMS SE). 2. – 5. Submitted for the purpose of renewal.
Material and methods:	
Test material:	^{14}C-Thiophanate-methyl
Lot/Batch No:	EC-01-02
Radiochemical purity:	98.0%
Test concentration:	0.2 mg/l
Test system and conditions:	The route and rate of degradation of ^{14}C-thiophanate-methyl was investigated in two equilibrated water/sediment-systems. The water/sediment systems sampled from a river (Rhein river/Rheinsulz AG/Switzerland) and from a pond (Ormalingen BL/Switzerland), consisted of natural water filtered through a 0.2 mm sieve and the uppermost 5-10 cm of the sediment sieved through a 2 mm mesh. The experiment was performed in a gas flow-system in 1000 ml glass metabolism flasks (inner diameter: about 10.6 cm, area about 88.2 cm²). Sediment was added to a depth of about 2.5 – 3.5 cm, corresponding to a wet weight of 250 g for both river and pond sediment. The respective water (550 ml) was added to the sediments to reach a depth of about 6 cm. The flasks were connected to a trap containing only water during the equilibration (21 d) to moisten incoming air. After treatment with the test item (0.205 mg a.s./l) the flasks were additionally connected to a series of traps, the first trap contained 50 ml ethylene glycol and the second trap 50 ml 2N NaOH in order to trap volatile compounds. The samples were incubated at 20°C in the dark. The radiolabelled test item was added to the water surface. This concentration corresponded to an application rate of 600 g a.s./ha, assuming uniform distribution within a 30 cm water layer. Single samples were taken for analysis at days 0, 1, 2, 8, 16, 30, 58 and 100 for both systems. Additional samples were taken from the pond system after 140, 202, and 301 days of incubation.
Method of analysis:	The water phase was separated from the sediment using a pipette. The radioactivity content was measured with LSC. The samples where further analysed with HPLC. If the radioactivity level was too low the samples were concentrated under reduced pressure and analysed by HPLC and TLC. The sediments including the water that was left after pipetting were extracted with: a) Acetonitrile:water (8:2; v/v) up to three times b) Water, once (if necessary) c) Soxhlet extraction with acetone:water (9:1; v/v) except samples on day 0, 1 and 2. The radioactivity in the individual extracts was quantified by LSC. The extracts (a-c) were combined and concentrated under reduced pressure and thereafter analysed by HPLC. The radiocarbon content of the sediments was determined by LSC after combustion. Reference compounds: DX-105, FH-432, MBC (carbendazim), AV-1951, 2-AB, 3-

	OH-TM, 4-OH-TM and 3-OH-TM-S.
Kinetic evaluation:	Degradation rates were determined in the study. These results are now superseded by the kinetic re-evaluation performed in accordance with FOCUS (2006, 2014) (Kiesel and Geibel, 2015i and j). During peer review the applicant was requested to submit degradation rates for the water/sediment systems for metabolites 4-OH-TM and 2-AB considering only the data from maximum occurrence onwards (Evaluation table, Data requirement 4.8). These data were provided in Moshenberg and Drechsler (2017a,b).

Table 77: Water and sediment characteristics.

Parameters	River sediment	Pond sediment
-	Sandy loam	Clay loam
sand [%]	61.05	38.35
silt [%]	27.65	34.50
clay [%]	11.30	27.15
org. C [%]	0.97	6.13
pH ($CaCl_2$)	7.83	7.61
CEC [mval/100 g dry sedim.]	7.59	21.52
Microbial Biomass [g microb. C/kg dry sediment]	0.16	0.66

Results (tables copied from Addendum 7 to the Monograph of 13 November 1997 (2003).

During incubation, the oxygen content in water samples varied from 4.2 to 8.3 mg/l in the pond system and from 4.5 to 8.2 mg/l in the river system. The redox potential in water ranged from 115 to 210 mV in the pond system and from 140 to 203 mV in the river system. In the sediment the redox potential varied between – 77 and -161 mV in the pond system and between -108 and -205 mV in the river system, confirming reductive conditions. The pH in the pond system varied between 7.67 and 8.38 and between 7.86 and 8.42 in the river system.

The material balance for the pond and river test systems after treatment with ^{14}C-thiophanate-methyl is given in the tables below.

Table 78: Mass balance for Pond water/sediment system. As % of applied radioactivity, single replicates.

Fraction	Incubation time (d)							
	0	**1**	**2**	**8**	**16**	**30**	**58**	**100**
Water	105.9	87.0	80.0	43.4	30.9	20.1	10.2	3.3
Sediment	1.2	15.8	25.3	55.9	65.7	77.1	87.5	89.4
$^{14}CO_2$	-	<0.1	<0.1	<0.1	0.1	0.2	0.7	1.3
volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	107.1	102.8	105.2	99.3	96.7	97.5	98.5	94.0

Table 79: Mass balance for Pond water/sediment system. As % of applied radioactivity, single replicates.

Fraction	Incubation time (d)							
	0	**1**	**2**	**8**	**16**	**30**	**58**	**100**
Water	101.6	94.9	86.9	51.1	42.5	32.0	10.1	5.5
Sediment	0.8	6.8	14.6	49.0	58.5	66.7	84.1	84.2
$^{14}CO_2$	-	<0.1	<0.1	0.2	0.4	0.9	1.0	1.6
volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total	102.5	101.6	101.5	100.3	101.4	99.6	95.3	91.3

Carbendazim (MBC) was the major metabolite in both systems. Other metabolites detected were: 4-OH-TM, 2-AB, M10 and AV-1951. 4-OH-TM had a max concentration of 8.6% AR in the pond system and 9.5% AR in the river system; 2-AB, which appeared later (day 30 in the pond and day 16 in the river system) with a max conc. of 7.5% AR in the pond system and 6.5% in the river system. The max concentration of M10 was 9.3% AR in the pond system while the maximum concentration in the river system was only 3.2% AR. AV-1951 seemed to be of more transient nature and had a max conc. of around 6% AR in both systems. The tentative degradation products DX-105, FH-432, 3-OH-TM, and 3-OH-TM-S were not detected.

The non-extractable residues increased continuously in both systems and reached 70% AR after 301 days in the pond system and 48.1% after 100 days in the river system. The distribution of thiophanate-methyl and its degradation products in water, sediment (extract) and the total system, expressed as % of applied radioactivity is given in the tables below. The degradation pathway proposed by the author is shown in Figure 8.2.2.3-1.

Table 80: Characterisation of radioactivity in Pond water/sediment system. As % of applied radioactivity. Single samples.

Fraction	Sample	Incubation time (d)										
		0	**1**	**2**	**8**	**16**	**30**	**58**	**100**	**140***	**202***	**301***
Parent	Water	98.4	85.9	66.5	9.7	-	0.9	-	-	-	-	-
	Sedim.	-	2.0	6.0	8.1	6.1	7.4	-	-	-	-	-
	Total	98.4	87.8	72.5	17.9	6.1	8.3	-	-	-	-	-
MBC	Water	2.7	6.5	14.8	34.3	38.2	26.8	8.0	2.6	1.7	1.9	0.4
	Sedim.	-	2.4	5.5	28.4	36.9	36.7	46.5	30.2	18.3	18.7	4.7
	Total	2.7	8.9	20.3	67.2	75.1	63.5	54.5	32.8	20.0	20.5	5.1
4-OH-TM	Water	-	-	-	2.8	2.3	2.9	-	-	-	-	-
	Sedim.	-	-	0.2	2.3	5.0	5.7	4.6	1.4	1.5	0.6	0.5
	Total	-		0.2	5.1	7.3	8.6	4.6	1.4	1.5	0.6	0.5
AV-1951	Water	0.5	2.6	5.6	2.3	-	-	-	-	-	-	-
	Sedim.	-	0.4	0.6	1.5	-	1.8	-	-	-	-	-
	Total	0.5	2.9	6.1	3.8	-	1.8	-	-	-	-	-
2-AB	Water	-	-	-	-	-	-	0.5	-	-	-	0.3
	Sedim.	-	-	-	-	-	2.3	7.0	6.0	6.2	3.9	2.9
	Total	-	-	-	-	-	2.3	7.5	6.0	6.2	3.9	3.2
M10	Water	-	-	-	-	2.1	0.9	1.4	2.2	1.7	0.8	1.0
	Sedim.	-	-	-	-	2.4	2.9	5.3	6.1	7.7	7.7	6.1
	Total	-	-	-	-	4.5	3.8	6.7	8.3	9.3	8.5	7.1
$^{14}CO_2$		-	<0.1	<0.1	0.2	0.4	0.9	1.0	1.6	4.7	2.5	5.2
non extractable		0.2	2.0	1.9	5.1	6.2	8.9	20.7	40.6	48.5	52.1	70.0
Total		101.8	101.6	101.5	100.3	101.4	99.6	95.3	91.3	92.1	90.9	95.2

* Data from these sampling days were not presented in Addendum 7 to the Monograph of 13 November 1997 (2003).

Table 81: Characterisation of radioactivity in River water/sediment system. As % of applied radioactivity. Single samples.

Fraction	Sample	Incubation time (d)							
		0	**1**	**2**	**8**	**16**	**30**	**58**	**100**
Parent	Water	103.2	69.2	48.4	-	-	-	-	-
	Sedim.	-	-	-	0.4	-	-	-	-
	Total	103.2	69.2	48.4	0.4	-	-	-	-
MBC	Water	2.8	14.4	25.3	39.0	27.3	17.3	9.4	2.3
	Sedim.	-	9.0	16.7	42.6	42.8	48.7	50.4	33.0
	Total	2.8	23.5	42.0	81.6	70.1	66.1	59.8	35.3
4-OH-TM	Water	-	-	-	4.4	1.3	1.4	-	-
	Sedim.	-	0.6	1.4	5.1	4.7	3.8	3.1	-
	Total	-	0.6	1.4	9.5	6.0	5.2	3.1	-

Fraction	Sample	Incubation time (d)							
		0	**1**	**2**	**8**	**16**	**30**	**58**	**100**
AV-1951	Water	-	3.4	6.3	-	-	-	-	-
	Sedim.	-	-	-	-	-	-	-	-
	Total	-	3.4	6.3	-	-	-	-	-
2-AB	Water	-	-	-	-	0.7	-	-	-
	Sedim.	-	-	-	-	5.8	4.8	4.7	4.8
	Total	-	-	-	-	6.5	4.8	4.7	4.8
M10 Unknown	Water	-	-	-	-	1.6	1.3	0.8	0.7
	Sedim.	-	-	-	-	-	-	2.2	2.4
	Total	-	-	-	-	1.6	1.3	3.1	3.2
$^{14}CO_2$		-	<0.1	<0.1	<0.1	0.1	0.2	0.7	1.3
non extractable		0.4	5.5	6.3	7.3	11.3	19.9	27.1	48.1
Total		106.3	102.8	105.2	99.3	96.7	97.5	98.5	94.0

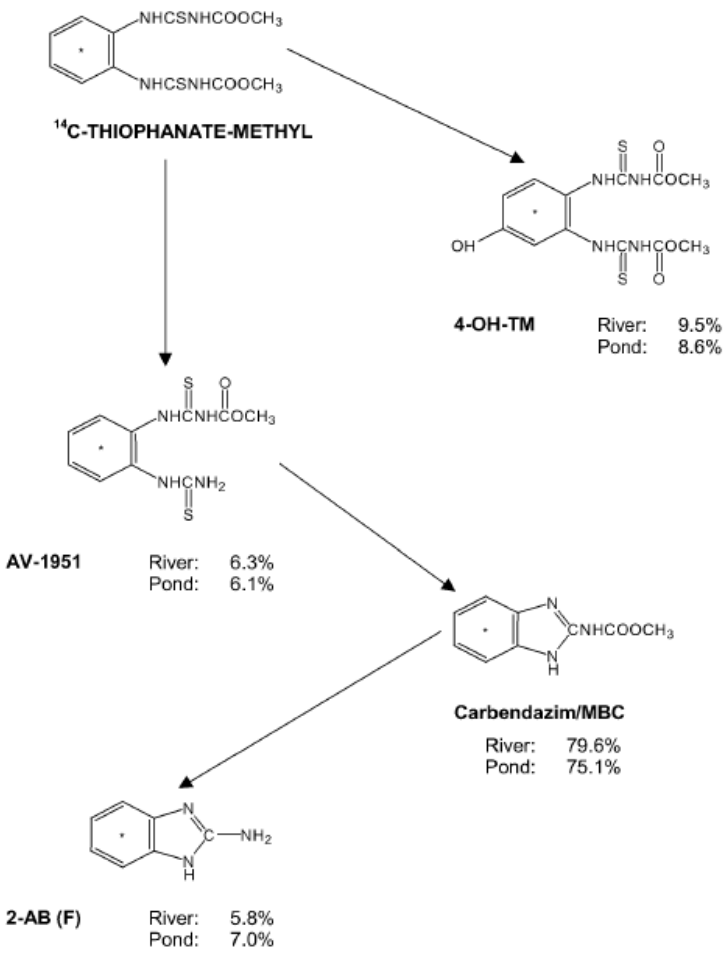


Figure 8.2.2.3-1. Proposed pathway for degradation in water/sediment systems (from Völkl, 2001). Note that some of the percentages of individual metabolites were not correct.

The degradation kinetics of thiophanate-methyl was re-evaluated by Kiesel and Geibel (2015i,j) based on the data from Völkl (2001). The assessment was conducted according to the FOCUS degradation kinetics guidance (2006, 2014) using the model KinGUI 2.1. The outcome is summarised in the table and figures below (though graphs are only shown for results that are discussed further, and not for dissipation in sediment since first of all the kinetic assessment gave unreliable results and secondly, these results are seldom used).

Degradation kinetics for thiophanate-methyl was calculated at the PI level for the total system for both the pond and river systems as was dissipation from the water phase. Dissipation from the sediment was only calculated for the pond system as not enough data points were observed from the maximum occurrence onwards in the river system.

The best fit for data from both the pond and the river water/sediment system was achieved with the SFO model for the total system.

Formation and degradation of the metabolites MBC, 4-OH-TM, 2-AB and M10 were calculated for the total systems at level MI. Metabolite M10 was at first considered as a primary metabolite but the estimated parameters did not reflect the measured values adequately. The author suggested that this may have been caused by the fact that most of parent had disappeared before the maximum occurrence of M10 was measured. In a second optimisation, a dummy compound was therefore introduced as a primary metabolite and M10 added as a secondary metabolite. The results for 4-OH-TM were used as input data for the dummy compound since the maximum occurrences of M10 and 4-OH-TM were similar and the max occurrence of 4-OH-TM was earlier than the one for M10. The Chi2 error-% for the dummy compound in this pathway fit (parent → dummy → M10) was 13.8% and p-value was 0.001. For 4-OH-TM (river system) the lower CI was marginally below zero but the p-value was acceptable, however due to high Chi2 error-% and poor visual fit the RMS did not accept the result. The Chi2 error-% was high for 4-OH-TM also in the pond system but here the visual fit was better so the result was considered acceptable by the RMS. For 2-AB and M10 the half-lives were estimated only for the pond system, as it was not possible to calculate half-lives for these metabolites in the river system. The results for 4-OH-TM and 2-AB are discussed further below since additional data were submitted during peer review.

Table 82: Kinetic parameters and the resulting DT50/90 values for thiophanate-methyl and its metabolites carbendazim (MBC), 4-OH-TM, 2-AB and M10 for the total water/sediment systems. Results for thiophanate-methyl are from parent only fits, other results are from pathway fits. From kinetic evaluation in Kiesel and Geibel (2015i,j) based on data in Völkl (2001).

Substance	System	kinetic model	Mo	χ2, % error	parameter	Confidence interval		Prob. > t	DT_{50} (days)	DT90 (days)
						lower	upper			
Thiophanate-methyl	Pond	SFO	105.18	6.7	k=0.199	0.16	0.24	10×10^{-5}	3.5	11.6
MBC		SFO-SFO	0	8.5	$k=9.09 \times 10^{--3}$	7.4×10^{-3}	0.011	9.8×10^{-13}	76.2	253.2
4-0H-TM		SFO-SFO	0	33	$k=1.22 \times 10^{-3}$	5.2×10^{3}	0.019	0.0008	56.7	188.4
2-AB		SFO-SFO	0	22	$k=1.27 \times 10^{-3}$	5.1×10^{-3}	0.020	0.001	54.7	181.8
M10 *		SFO-SFO	0	12	$k=1.45 \times 10^{-3}$	-7.0×10^{-4}	0.004	0.10	478.5 [a]	>1000
Thiophanate-methyl	River	SFO	108.39	3.3	k=0.424	0.38	0.47	2.1×10^{-4}	1.6	5.4
MBC		SFO-SFO	0	6.4	$k=7.6 \times 10^{-3}$	0.0051	0.010	1.2×10^{-5}	91.6	304.2
4-OH-TM		SFO-SFO	0	38	$k=0.0182$	-1.3×10^{-3}	0.038	0.043	38.2 [a]	126.8

* The results for M10 are from separate pathway fit parent → dummy → M10.

a Results not considered acceptable by the RMS.

Table 83: Summary of formation fractions optimised in Kiesel and Geibel (2015i,j) based on data in Völkl (2001), for the total systems pathway fits.

Degradation pathway	Kinetic model	System	Formation fraction	Standard deviation
Thiophanate-methyl → MBC	SFO-SFO	Pond	0.81	0.061
		River	0.76	0.044
MBC→ 2-AB		Pond	0.25	0.063
Thiophanate-methyl → 4-OH-TM		Pond	0.08	0.012
		River	0.07 [a]	0.016
Thiophanate-methyl → Dummy		Pond	0.10	0.017
Dummy → M10 *		Pond	1.00 [a]	0.24

* The results for M10 are from separate pathway fit parent → dummy → M10.

a Results not considered acceptable by the RMS.

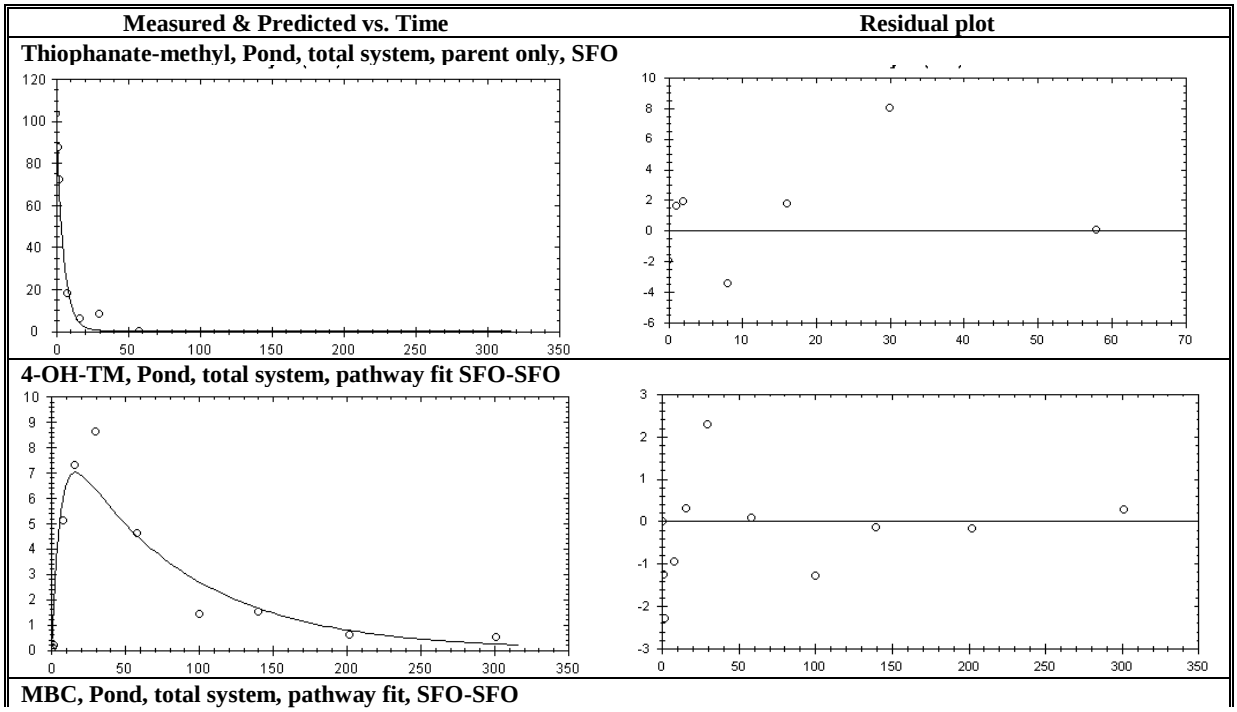
Table 84: Water dissipation rates, summary of kinetic re-evaluation, from Kiesel and Geibel (2015i,j).

Substance	System	kinetic model	Mo	χ2, % error	parameter	Confidence interval		prob > t	DT_{50} (days)	DT_{90} (days)
						lower	upper			
Thiophanate-methyl	Pond	SFO	106.3	5.0	k=0.250	0.21	0.29	1.7×10^{-4}	2.8	9.2
Thiophanate-methyl	River	SFO	108.1	3.2	k=0.425	0.36	0.49	0.0027	1.6	5.4

Table 85: Sediment dissipation rate, summary of kinetic re-evaluation, from Kiesel and Geibel (2015i,j).

Substance	System	kinetic model	Mo	χ2, % error	parameter	Confidence interval		prob > t	DT_{50} (days)	DT_{90} (days)
						lower	upper			
Thiophanate-methyl	Pond	SFO	8.45	26.6	k=0.027	-0.010	0.063	0.15	26.1 [a]	86.7

a Result not considered acceptable by the RMS.



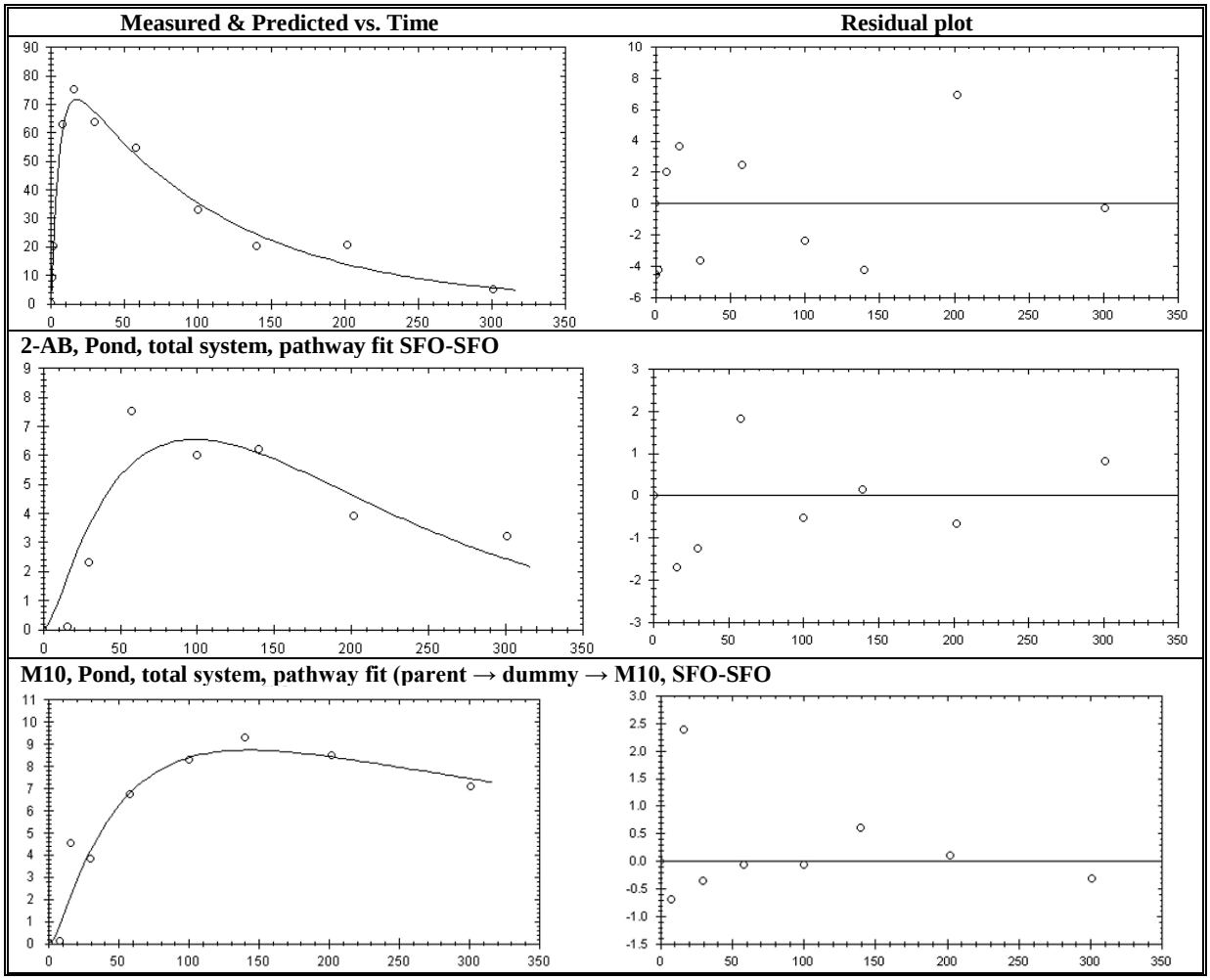


Figure 8.2.2.3-2. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl and metabolites in Pond total system (from Kiesel and Geibel, 2015i,j).

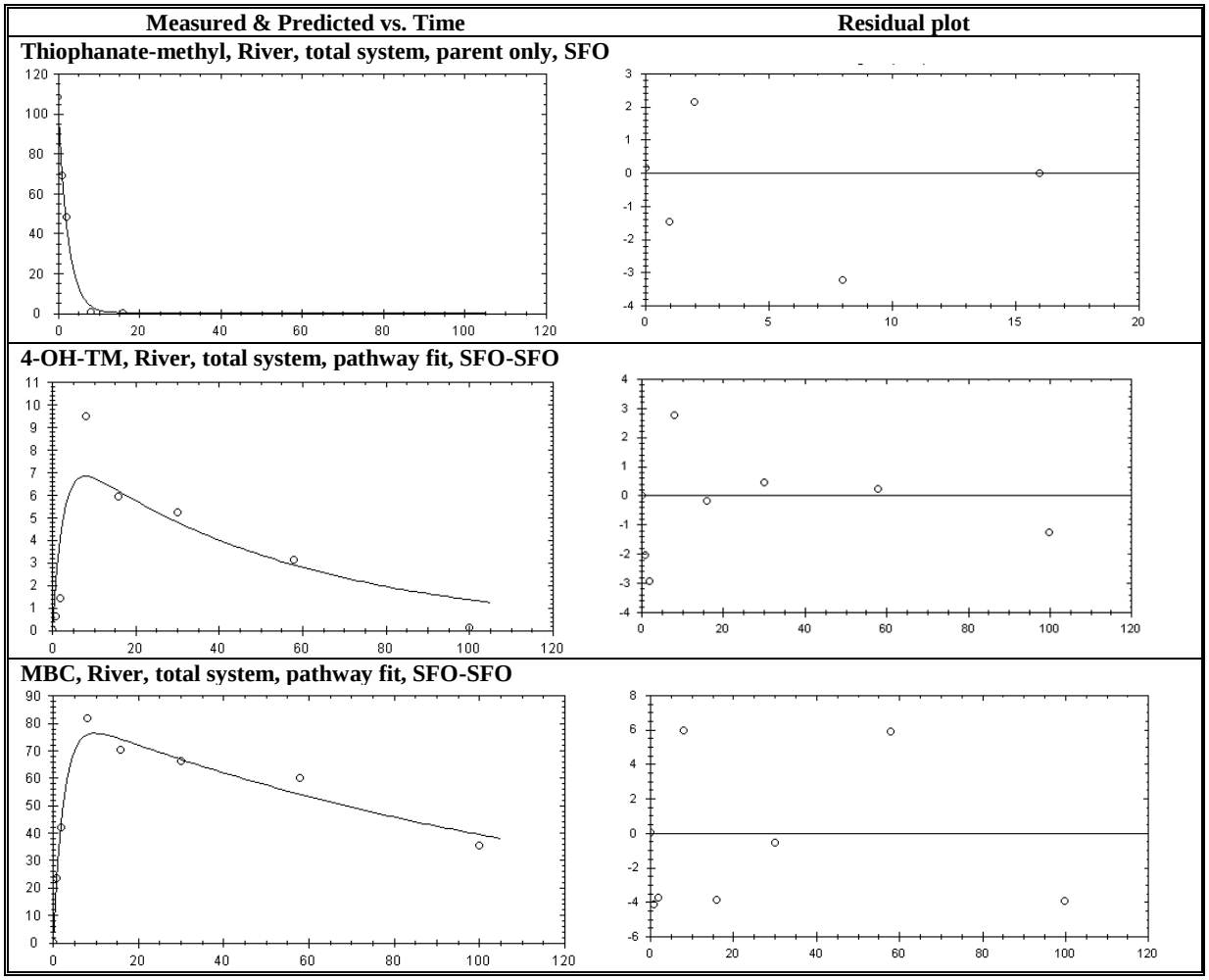


Figure 8.2.2.3-3. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl and metabolites in River total system (from Kiesel and Geibel, 2015i,j).

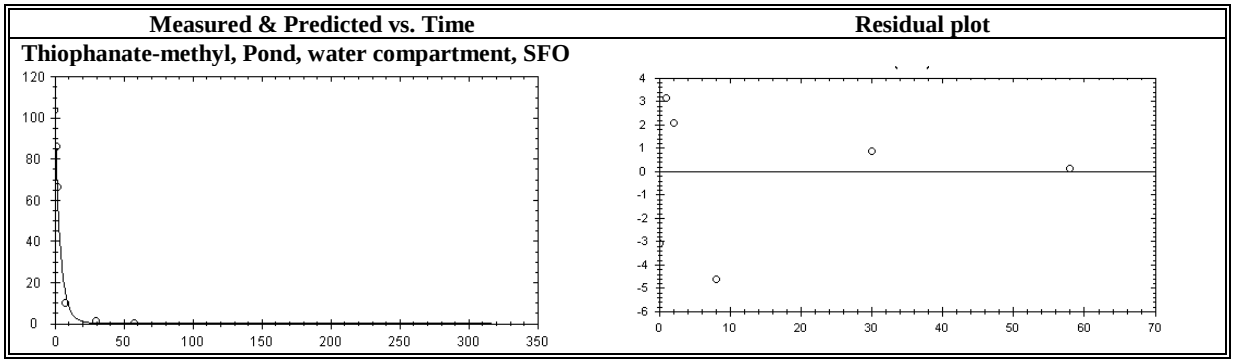


Figure 8.2.2.3-4. Visual fit and plot of residuals for kinetic fitting of data for Thiophanate-methyl in Pond water compartment (from Kiesel and Geibel 2015i,j)

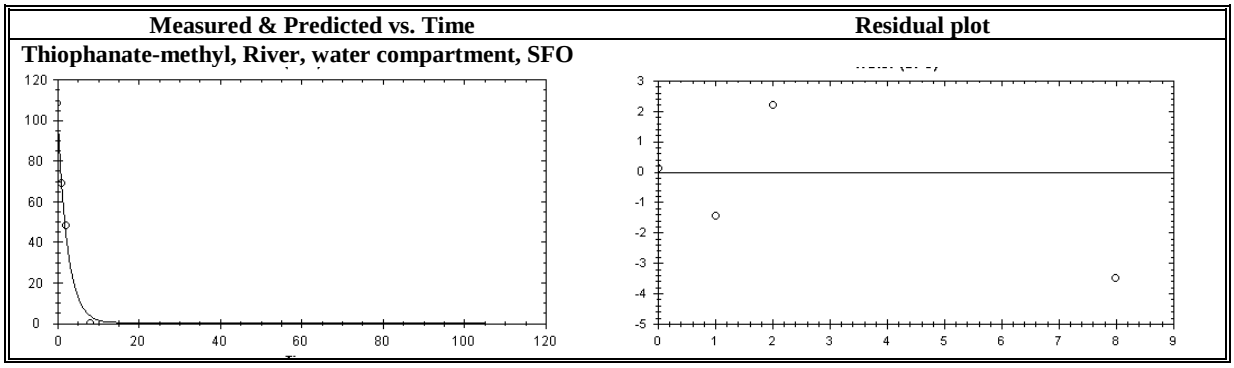


Figure 8.2.2.3-5. Visual fit and plot of residuals for kinetic fitting of data for thiophanate-methyl in River water compartment (from Kiesel and Geibel, 2015i,j).

During peer review the above results for 4-OH-TM and 2-AB in the pond system were questioned since Chi2 error-% were relatively high and since the peak was not covered by the fit. Kinetic evaluation using only data from the maximum observed onwards was therefore requested (Evaluation table, Data requirement 4.8). In response, Moshenberg and Drechsler (2017a,b) submitted such optimisations for 4-OH-TM for both water/sediment systems, and for 2-AB for the pond system (no data were presented for 2-AB in the river system presumably since only 4 data points were available from the peak observation). SFO and FOMC kinetic models were run in KinGUI 2.1. The first data point with no residues was set to ½LOQ (0.1% AR). The SFO endpoints were considered acceptable, and the authors suggested that these could be used for modeling as well as trigger endpoints. The RMS agrees to the conclusions.

Table 86: Kinetic parameters and the resulting DT50/90 values for metabolites 4-OH-TM and 2-AB using only data from max occurrence onwards (decline from peak). Total water/sediment systems. From kinetic evaluation in Moshenberg and Drechsler (2017a,b) based on data in Völkl (2001).

Substance	System	kinetic model	Mo	χ2, % error	parameter	Confidence interval		Prob. > t	DT_{50} (days)	DT90 (days)
						lower	upper			
4-0H-TM	Pond	SFO	8.52	12.0	k=0.02136	0.02	0.03	8.1E-04	32.4	108
		FOMC	8.65	9.8	α= 2.32 ß= 80.5	-0.8 -63	5.4 225	n.r. n.r.	not reliable	not reliable
2-AB		SFO	7.45	6.3	k=0.003662	0.002	0.005	0.006	189	629
		FOMC	7.46	7.2	α= 19.5 ß= 5220	-1098 -3.0E+05	1137 3.1E+05	n.r. n.r.	not reliable	not reliable
4-OH-TM	River	SFO	8.80	13.2	k=0.02641	0.01	0.04	0.01	26.2	87.2
		FOMC								

n.r. Not relevant.

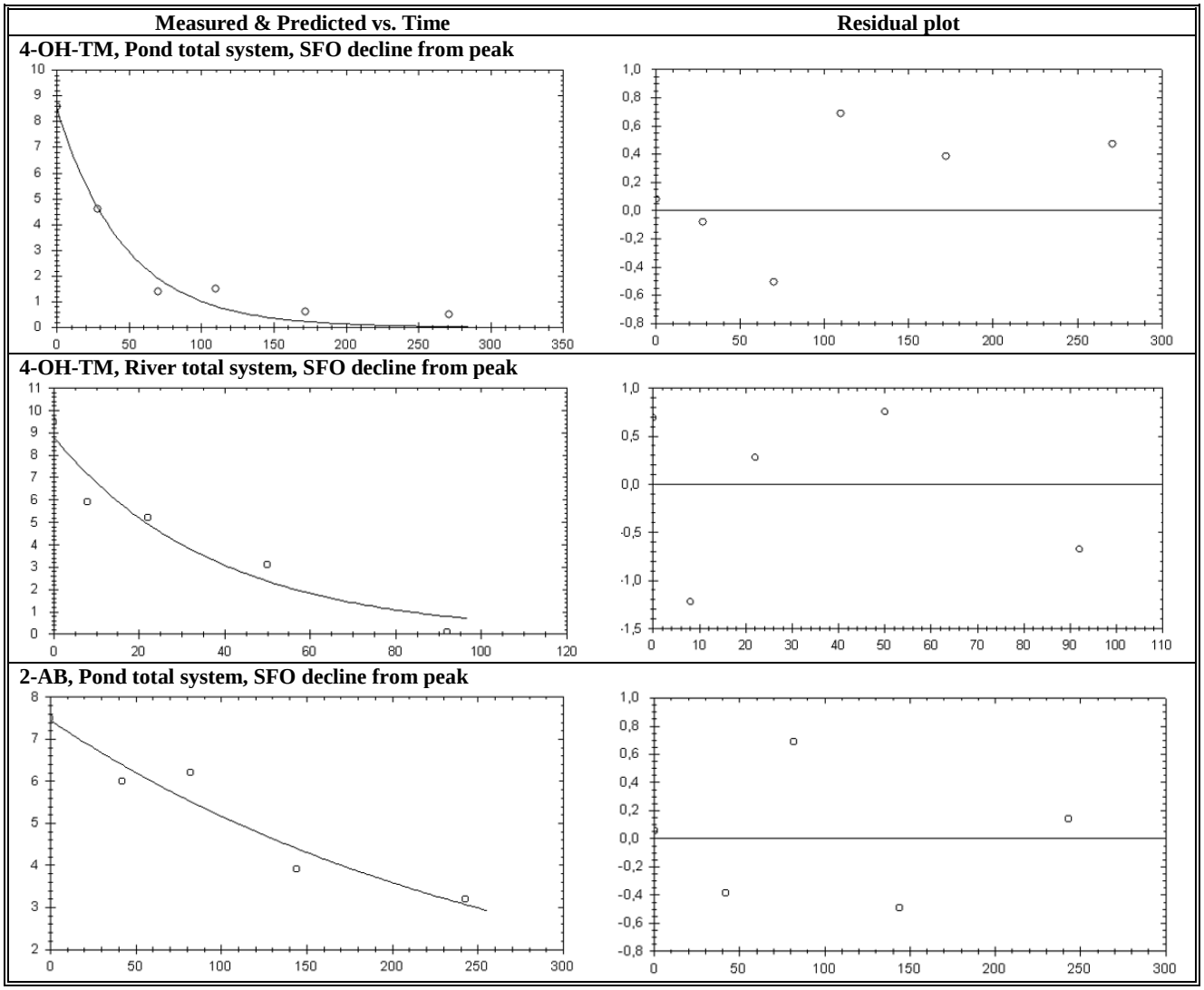


Figure 8.2.2.3-6. Visual fit and plot of residuals for kinetic fitting of data for 4-OH-TM in Pond and River total systems, and for 2-AB in Pond total system. As decline from peak observation. (from Moshenberg and Drechsler (2017b)

RMS comments and conclusion

The water/sediment study was accepted in the Addendum (2003) to the DAR (1997) and it is considered acceptable also for the purpose of renewal. Most of the kinetic evaluation is accepted**.** The derived endpoints for metabolite M10 is however not accepted due to the fact that the derived degradation rate constant was not statistically significant different from zero. Also the results for 4-OH-TM from the river system presented in Kiesel and Geibel (2015i,j) are considered as unreliable, since the fit was poor visually and since the Chi2 error-% was high (the lower confidence interval was below zero however the t-test indicated that the result was acceptable so this is not considered as a reason for omitting the endpoints). The $DisT_{50}$ in sediment calculated for the parent for the pond system is not considered reliable (t-test failed). Besides this a few errors in the kinetic report has been noted. Kiesel and Geibel (2015i,j) stated that metabolite M10 was only detected in the pond system. This was not correct but the RMS accepts that no kinetic evaluation was submitted for M10 for the river system, since it is not considered likely that useful results would have been obtained. Furthermore, Kiesel and Geibel (2015i,j) claimed that the half-lives for 2-AB and M10 are geometric means when in fact they were single values. Finally, the day 0 value entered for thiophanate-methyl was higher than the total mass

balance for each system. However, the deviations were not large (+1.4 and +1.9% AR for the pond and river system, respectively). Finally, the endpoints presented for 4-OH-TM and 2-AB by Moshenberg and Drechsler (2017a,b) are considered acceptable and the RMS suggests that those results should replace the corresponding results presented by Kiesel and Geibel (2015i,j). The requirement in Evaluation table, Data requirement 4.8 is considered as fulfilled.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

Reference:	**1. Knoch, E. (2001)** Degradability and fate of (U-^{14}C-phenyl) carbendazim in the aquatic environment (water/sediment system) **2. Zillgens, B. (2007c)** Predicted environmental concentrations of carbendazim in surface water and sediment associated with uses in arable crops: Modeling for the European Union
Report No.:	1. C017201, WAS2002-167 2. DuPont-23452
Guideline:	SETAC Europe, Dutch guideline
GLP:	1. Yes
Validity	1. Valid

Material and methods:

Two test systems were chosen according to the recommendations of the OECD Belgrate Workshop that differed significantly in their characteristics. The data are reported in the table below.

The metabolism flasks were filled with a layer of water-saturated sediment (1 cm for sediment Bickenbach, 2 cm for sediment Unter Widdersheim). The height of the sediment layers corresponded to approximately 95 g (Bickenbach) and 120 g (Widdersheim), respectively. The water column height corresponded to about 6 cm (approximately 300 ml of water). The sediment dry weight to water ratio was approx. 1:4 (w:w) for both test systems. Air entering the system by pump suction was passed through a washing bottle filled with water. Water/sediments were aerated and slightly agitated from the top by means of suspended magnetic stirrer. Air leaving the test system was successively passed through sodium hydroxide and 2-methoxy ethanol traps to collect $^{14}CO_2$ and other organic volatiles, respectively. [U-^{14}C-phenyl] Carbendazim was applied onto the water surface of each sample. Each water phase was treated at 0.2 ppm level (equivalent to approximately 60 µg [U-^{14}C-phenyl] carbendazim/300 ml water). A further exaggerated rate was applied at 2.0 mg/l ^{14}C-carbendazim, installed for possible metabolite identification. The experiment was carried out in the dark with single samples at each sampling point and radioactive treated water/sediment system at 20°C. Water and sediment were analysed separately at the following times: zero time, 3, 7, 14, 21, 28, 42, 62, 76, 98, 120 and 149 days after application. Sediment samples were extracted several times by shaking with 50 to 100 ml of acetonitrile:water (4:1; w:w). The remaining residual radioactivity in sediments were assayed by combustion.
The total recoveries of radioactivity ranged from 93.3 % to 105.9 % of the total applied radioactivity.

Kinetic analyses: DT_{50}/DT_{90} were presented in the original report (Knoch, 2001). The results were also re-calculated using TopFit model (Kley, 2002) but those results are not re-produced here because the DT_{50}/DT_{90} were also re-calculated in accordance with FOCUS guidance (in Zillgens, 2007c) and those results were the final ones.

Table 87: Physico-chemical characterisation of water and sediment systems before incubation with [U-^{14}C-phenyl]-carbendazim.

Parameters	Bickenbach-System		Unter Widdersheim-System	
	Water	Sediment	Water	Sediment
pH	8.5	8.0	8.1	7.5
Redox pot. [mV]	167	136	157	184
CEC [meql/100 g]		11.1		127
Org. C [%]		0.41		2.28
Microbial act. (start) [mg C/100 g]		18		22
Dry weight [%]		80.1		63.9
Sediment texture (USDA)		sand		loam
Sand [%]		93.1		41.7
Silt [%]		2.0		37.0
Clay [%]		4.6		21.3

Results

The formation of $^{14}CO_2$ accounted for 6.0 to 23.0% AR by the end of the study. Other volatile degradation products were observed at equal or less than 0.1% of applied dose during the experiment.

The balance and distribution of the applied radioactivity (% applied) is given in the tables below.

Table 88: Distribution of applied radioactivity in Bickenbach water/sediment-system during incubation with [U-^{14}C-phenyl]-carbendazim (% AR).

Fraktion	Incubation time (days)						
	0	3	14	28	62	98	120
Water	99.0	85.5	44.4	24.0	11.0	8.4	9.1
Sediment (extractable)	4.2	13.7	17.2	14.9	8.1	5.8	9.0
Sediment (unextractable)	0.3	4.4	39.5	62.5	63.4	61.0	55.2
Sediment total	4.5	18.1	56.7	77.4	71.5	66.8	64.2
CO_2	-	<0.1	0.5	2.7	13.0	18.1	20.4
Volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mass balance	103.5	103.6	101.6	104.1	95.5	93.3	93.7

Table 89: Distribution of applied radioactivity in Unter Widdersheim water/ sediment-system during incubation with [U-^{14}C-phenyl]-carbendazim (% AR).

Fraktion	Incubation time (days)						
	0	3	14	28	62	98	120
Water	103.2	55.6	30.4	9.7	4.7	3.8	3.4
Sediment (extractable)	2.0	37.8	64.7	73.2	55.6	42.5	28.5
Sediment (unextractable)	0.7	10.4	9.3	19.1	33.8	46.4	59.4
Sediment total	2.7	48.2	74.0	92.3	89.4	88.9	87.9
CO_2	-	<0.1	0.2	.6	2.0	3.7	4.7
Volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Mass balance	105.9	103.8	104.6	102.6	96.2	96.5	96.1

The test substance carbendazim was degraded in both test systems. The initial step of carbendazim degradation resulted in the formation of 2-aminobenzimidazole (AEF033008). 1,2-Phenylendiamine (AE F037197), a possible next step in degradation, was not found in any compartment of the aerobic aquatic model systems. The overall conversion was accompanied by the formation of non-extractable residues and mineralisation to $^{14}CO_2$.

Table 90: Distribution of parent and metabolites in Bickenbach and Unter Widdersheim system during incubation with carbendazim (% AR).

Component	Analysis time (days)							
	0	**3**	**7**	**14**	**28**	**62**	**98**	**120**
Bickenbach								
Water								
Carbendazim	99.0	97.1	67.0	35.9	13.3	0.8	0.4	0.2
1-Amino benzimidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1.2-Phenylendiamine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Unknown fractions	<LOD	<LOD	<LOD	U1:6.9	U1:9.8	U1:7.8 U3:0.7 U4:1.0 U6:0.9	U1:6.2 U3:0.7 U4:0.5 U6:0.3	U1:2.4 U2:2.6 U4:4.3 U5:3.0 U6:5.5
Sediment extracts								
Carbendazim	n.a.	13.0	14.3	14.9	9.4	5.3	3.7	3.8
1-Amino benzimidazole		<LOD	<LOD	<LOD	<LOD	0.9	0.4	1.1
1.2-Phenylendiamine		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Unknown fractions		<LOD	<LOD	<LOD	U1:2.5	U1: 1.1	<LOD	<LOD
Unter Widdersheim								
Water								
Carbendazim	103.2	54.7	36.8	28.7	7.9	3.5	1.1	n.a.
1-Amino benzimidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	n.a.
1.2-Phenylendiamine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	n.a.
Unknown fractions	<LOD	<LOD	<LOD	<LOD	<LOD	U1:0.7 U4:0.5	U1:2.4	n.a.
Sediment extracts								
Carbendazim	n.a.	36.7	51.7	61.8	68.0	50.9	37.9	24.8
1-Amino benzimidazole		<LOD*	<LOD*	<LOD*	<LOD*	3.3 6.3(76 d)	4.8	3.1
1.2-Phenylendiamine		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Unknown fractions		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

LOD between 0.2% AR and 5% AR

*LOD of 2.5% AR

At zero-time, ^{14}C-carbendazim was observed in whole test systems at 99.0 and 103.2% of the applied dose decreasing to <1.0 and 33.4% AR (Bickenbach/Widdersheim respectively) at the end of the study, day 149. The metabolite 2-aminobenzimidazole was detected at a maximum peak value of 6.3% AR in the whole system of Unter Widdersheim after 76 days of incubation. A maximum of six unknown metabolites were assigned in the chromatographed radioactivity.

Radiolabeled carbendazim was present in all water phases analyzed. The initial values of the test substance (99.0 to 103.2% AR) decreased to a minimum value of 0.5% AR on day 149.

The presence of 2-aminbenzimidazole in the water phases of both test systems was reflected by peak values below 2.5% of the applied dose. A maximum of six unknown metabolites were assigned in the chromatographed radioactivity. After 3 days of incubation radiolabeled carbendazim was found with 13.0 and 36.7% AR in the sediment extractable radioactivity. A maximum peak value of 68% AR was observed in the sediment on day 28. After 5 months of incubation ^{14}C-carbendazim decreased to <5 % and 33.4% AR in the sediment extractable radioactivity of ”Bickenbach” and ”Unter Widdersheim” location, respectively. In the sediment extracts unknown metabolites reached

radioactivity levels clearly below 5% of the applied radioactivity. 2-Aminobenzimidazole yielded for a peak maximum of 6.3% AR in the sediment extractables on day 76 not exceeding 5% in two consecutive time points.
The non-extractable residues were subjected to an organic matter fraction of the sediments. With a value of 32.1 to 40.8% AR, most of the residual radioactivity was found in the humin fraction after 98 to 120 days of incubation.
Carbendazim was degraded in both aquatic test systems. The half-life for parent compound along with their disappearance times (DT-90) and their errors in the water phases and the total systems are summarised in the below table. The calculations were carried out by using the software EXCEL and based on a single-exponential first-order kinetics as the mathematical model.

Table 91: DT_{50} and DT_{90} for carbendazim in the two water/sediment systems.

System	Disappearance time					
	From total systems			From water		
	DT-50 (d)	DT-90 (d)	r^2	DT-50 (d)	DT-90 (d)	r^2
Bickenbach	16.1	53.5	0.9893	10.8	36.0	0.9947
Widdersheim	73.6	244.4	0.9940	5.8	19.2	0.9651

The DT_{50} for both systems were recalculated regarding FOCUS Kinetics Guideline (2006) and documented as in the below table.

Table 92: Re-calculation according FOCUS Kinetics Guideline (2006) (Zillgens, 2007c).

System	Disappearance time from total systems			
	DT50 (d)	DT90 (d)	χ2error	Model
Bickenbach	15.1	50	11	SFO
Widdersheim	75.2	249.7	12	SFO

Conclusion

The study is valid and plausible.

Reference:

1. **Völkl, S. (2002)** ^{14}C-Thiophanate-methyl: Degradation and Metabolism in Three Soils Incubated under Aerobic Conditions.
2. **Kiesel, A. and Geibel, E. (2015a)** Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014).
3. **Kiesel, A. and Geibel, E. (2015b)**. Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on a laboratory study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier – M-CA Section 7.
4. **Kiesel, A. and Geibel, E. (2016a)** Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014).
5. **Kiesel, A. and Geibel, E. (2016b)**. Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on a laboratory study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier – M-CA Section 7.
6. **Kiesel, A., Drechsler, S., Geibel, E. (2017a)** Assessment of degradation kinetics of Thiophanate-methyl in three soils under laboratory conditions according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014)
7. **Kiesel, A., Drechsler, S., Geibel, E. (2017b)** Raw data to the assessment of

	degradation kinetics for Thiophanate-methyl based on data of a laboratory soil study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7
Report No.:	1. 815051 2. RD-03326 3. RD-03369 4. RD-03326N 5. RD-03369N 6. RD-03326N2 7. RD-03369N2
Document No.:	1. 721-004 2. 782-035 3. 782-041 4. 782-088 5. 782-089 6. 782-097 7. 782-098
Guideline:	1. OECD TG No. 307 (draft August 2000); US EPA 162-1 2. - 7. FOCUS (2006, 2014)
GLP:	1. Yes 2. - 7. Not applicable
Previous evaluation:	1. In Addendum (2003) to DAR (1997) 2. - 7. Submitted for the purpose of renewal
Material and methods:	
Test material:	Phenyl-UL-^{14}C-labelled thiophanate-methyl
Lot No:	EC-01-01 (RCC Ltd Code: 115065/A, 116973/A)
Radiochemical purity:	98.9% (determined by HPLC)
Test system/test conditions:	In Völkl (2002), the route and rate of degradation of thiophanate-methyl under aerobic conditions was studied in three soils. 100 g dry soil were filled into all-glass metabolism flasks. Soil samples were treated with ^{14}C-thiophanate-methyl dissolved in acetone, at nominally 1.48 mg a.s./kg dry soil, which corresponded to 1.54–1.86 kg a.s./ha assuming 10 cm mixing depth and the soil's dry bulk densities. The soils were incubated in the dark at 20±2ºC at 46% MWHC. Flow-through systems with absorption traps with ethylene glycol and 2N NaOH for VOC and $^{14}CO_2$, respectively, were used. Duplicated samples were taken after 0, 1, 3, 7, 14, 28, 56, 120 days of incubation. For day 0, no adsorption traps were used and only one extraction with acetonitrile at room temperature was performed. For all other sampling intervals, the samples were subject to extraction with acetonitrile:water (8:2; v/v) at room temperature up to four times and one Soxhlet extraction with acetonitrile:water (9:1; v/v). On day 120, an additional reflux extraction was performed for 4 hours with acetonitrile:2M hydrochloric acid (8:2; v/v). The radioactivity in all extracts was measured with LSC. Non-extractable radioactivity was quantified by LSC after combustion. The LOQ of the LSC counter was 0.06% AR. An additional test with Soxhlet extraction was performed to proof that radioactive fractions M1 and M2 were not formed during the extraction step. Therefore, one soil sample of soil III (Speyer 2.3) was fortified with ^{14}C-thiophanate-methyl and extracted by Soxhlet extraction as described above and analysed by HPLC.

The extracts were pooled, concentrated and then chromatographically profiled with HPLC to determine parent (thiophanate-methyl) and its main metabolites with a LOQ of 0.3% AR. For the purpose of metabolite identification, the following reference standards were used: DX-105, FH-432, CF-27 (Carbendazim, MBC), AV-1951, 2-AB, 3-OH-TM, 4-OH-TM, 3-OH-TM-S. TLC was used as confirmatory method on selected soil samples.

Soil organic matter fractionation was carried out on a sample from day 120 (after the additional harsh extraction).

The presence of $^{14}CO_2$ in the NaOH-traps was confirmed by $BaCO_3$-precipitation. Microbial biomass was determined before treatment and after about 127 days of incubation. It corresponded to at least 1% of the organic carbon content of the soils.

In Völkl (2002), DT_{50}s were calculated for thiophanate-methyl and the metabolites carbendazim and the unknown M2 using first-order kinetic model (non-linear curve fitting). These results were superseded by the re-evaluation by Kiesel and Geibel (2015a,b) which during the evaluation was replaced by Kiesel and Geibel (2016a,b). The kinetic re-evaluation was performed according to FOCUS Degradation kinetics report (2006, 2014) with KinGUI version 2.1. Parent only data were fitted to SFO and FOMC kinetic models. Degradation pathway-fits were performed for thiophanate-methyl and the metabolites carbendazim, CM-0237 and 2-AB in a sequential fitting approach, using SFO-SFO. The optimization method IRLS and the optimization algorithm LM were used. The results were normalised to standard conditions (20°C and pF 2) to fulfil requirements for modelling endpoints according to FOCUS Groundwater (2000, 2014).
During peer review degradation rate for one additional soil was requested for the metabolite CM-0237 (Evaluation table, Data requirement 4.2). In response to the requirement, Kiesel, Drechsler & Geibel (2017a,b) estimated parameters for CM-0237 for the Bretagne soil (soil I). In the previous kinetic evaluations parameters were not estimated for CM-0237 in the Bretagne soil (soil I), since CM-0237 was only found in minor amounts in that soil.

Table 93: Soil characteristics.

		Bretagne (soil I)	**Mussig (soil II)**	**Speyer 2.3 (soil III)**
Origin		France	France	Germany
Soil plot history		No pesticide treatments for at least 5 years prior to soil sampling. No cultivation in these fields since 1995.		No pesticides between 1997 and 2001.
Collection and storage		Collected in September 2001. Stored for about 3 weeks at 4°C. Sieved (2 mm). Acclimation at 20°C for about 5 days before study.		Collected on July 20, 2001. Sieved (2 mm). Stored for about 8 weeks at 4°C. Acclimation at 20°C for about 5 days before study.
Soil type (USDA)		Silt loam	Clay loam	Sandy loam
Clay <2µm	[%]	19.5	34.2	9.0
Silt 2–50 µm	[%]	61.2	44.3	33.3
Sand >50µm	[%]	19.3	21.5	57.8
Dry bulk density	[g/cm^3]	1.04	1.04	1.26
Water content at pF 1.0 (MWHC)	[g/100 g soil]	60.5	66.0	38.0
40–60% MWHC	[g/100 g soil]	24.2-36.3	26.4-39.6	15.2-22.8
Water content at pF 1.8	[g/100 g soil]	43.0	45.7	27.7
Water content at pF 2.5	[g/100 g soil]	29.9	31.2	13.8
pH ($CaCl_2$)		5.8	7.5	6.5
Organic carbon	[g/100 g soil]	2.0	3.0	1.3
CEC	[meq/100 g soil]	8.5	36.2	10.0
Biomass – start of incubation	[mg/100 g soil]	23.5	34.1	21.3
Biomass – end of incubation	[mg/100 g soil]	18.7	36.5	13.9

Results

Mass balance and characterisation of thiophanate-methyl and its metabolites are listed in the tables below. The total recovery in individual replicates ranged from 93.3 to 102.9% AR for all soils and for all sampling intervals. The extractable radioactivity decreased from >98.6% AR at day 0 to 15.3–31.2% AR at the end of the study (day 120). Soxhlet extraction released a maximum of 22.3% AR. The additional harsh extraction performed with sample A at day 120 resulted in high amounts of additionally extracted material; up to 30.3% AR in soil III (5.6% and 15.3% AR in the other two soils). The non-extractable fractions increased continuously from 1.4–3.2% AR at day 0 to 39.7–73.2% AR at day 120. The organic matter fractionation resulted in a relatively equal distribution among fulvic acids, humic acids and humins in the silt loam (Bretagne, soil I) and the sandy loam (Speyer 2.3, soil III). The major part (85.5%) of the non-extractable radioactivity in the clay loam (Mussig, soil II) was bound to humins, 12% to fulvic acids and less than 2% to humic acids. $^{14}CO_2$ reached 7.3–7.6% AR in soils II and III and was 25.7% AR after 120 days in soil I. Other volatile compounds were below 0.1% AR at all sampling intervals and all soils. The microbial biomass measurements showed that the samples were viable throughout the study, although the microbial biomass decreased in two of the soils (Bretagne and Speyer 2.3).

Thiophanate-methyl was almost entirely degraded within the first 7 days. In all soils, the concentration of thiophanate-methyl was <0.5% AR after day 7 with no detection at the last two sampling intervals (day 56 and 120).

The route of degradation as proposed by Völkl (2002) was the same as in Adam (2014); see Figure 8.1.1.1-6.
The metabolite carbendazim was formed in all soils in large amounts already after 1 day (42.2–58.7% AR) and reached maxima of 62.8-75.8% in the different soils after 3 or 7 days, respectively. Two additional metabolites were detected in

concentrations ≥5% at a minimum of two consecutive sampling intervals in at least one soil: CM-0237 and 2-AB. The metabolite CM-0237 was characterised in Völkl (2002) by LC-MS analysis, but referred to as “M2” in the study, while the applicant referred to the metabolite as CM-0237 in documents M-CA, M-CP and N3.

Metabolite CM-0237 (M2) reached a maximum of 9.8% AR (after 3 days). This metabolite together with M1 (named CM-0238 by the applicant) were present as impurities in the application solution. The author, therefore, subtracted the mean amounts before and after application from the amounts of CM-0237 (M2) and M1 detected in soil extracts. These mean amounts were 1.3% in the case of CM-0237 (M2) and 2.7% in the case of CM-0238 (M1). This resulted in originally measured maximum concentrations of 11.1% and 7.7%. M1 was only detected once in a concentration ≥5%. Both metabolites were characterised in Völkl (2002) by LC-MS with molecular masses of 306 and 325 g/mol, respectively. The author furthermore showed that neither of the two metabolites was formed during extraction steps.

The metabolite 2-AB reached a maximum of 6.1% with standard extraction at day 14 in soil Mussig (soil II) and was 5.0% on average at the following sampling interval (day 28). A maximum of 18% AR was extracted with the additional harsh extraction at day 120 from soil Speyer 2.3 (soil III).

Additionally, the metabolite DX-105 and up to 9 unknown other metabolites were detected. None of them exceeded 4.7% AR.

Table 94: Distribution and characterisation of radioactivity in the Bretagne soil (soil I) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20±2ºC and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.* All values as % of applied radioactivity (AR).

	days after application							
	0	**1**	**3**	**7**	**14**	**28**	**56**	**120**
Parent	85.7	15.9	1.4	0.9	-	0.6	-	-
	81.1	23.5	1.7	4.6	-	-	-	-
Mean	83.4	19.7	1.6	2.7	-	0.3	-	-
Carbendazim	8.3	63.0	72.6	76.2	68.0	53.5	38.9	23.0
	10.2	54.5	74.2	75.4	69.1	50.8	36.7	25.2
Mean	9.3	58.7	73.4	75.8	68.5	52.1	37.8	24.1
DX-105	1.7	4.0	1.3	-	2.5	-	-	-
	1.6	4.6	1.5	-	-	-	-	-
Mean	1.6	4.3	1.4	-	1.2	-	-	-
2-AB	-	-	0.6	1.3	3.4	3.2	3.3	2.6
	-	-	0.4	-	3.7	2.9	3.2	2.8
Mean	-	-	0.5	0.6	3.6	3.0	3.3	2.7
CM-0237 (M2) [a]	1.3	3.6	2.3	1.3	1.0	0.3	-	-
	1.3	4.7	2.0	1.6	1.0	0.2	0.1	-
Mean	1.3	4.1	2.1	1.4	1.0	0.3	0.1	-
CM-0238 (M1) [a]	-	-	-	-	-	-	-	-
M3	-	-	1.7	1.9	1.2	0.7	0.5	0.3
M6	-	0.6	1.2	1.7	1.3	2.4	1.0	1.6
M7	0.7	0.8	0.6	-	0.5	0.3	0.5	0.3
M8	-	1.5	0.3	-	-	0.2	0.4	0.1
Extractable	98.6	86.0	77.1	69.3	57.5	47.7	31.6	10.5
Soxhlet	n.p.	6.6	8.6	16.2	21.9	12.9	14.4	20.7
Total extractable	98.6	92.6	85.7	85.5	79.4	60.6	46.0	31.2
Non-extractable	3.2	4.9	11.2	12.2	17.5	30.1	39.3	39.7
$^{14}CO_2$	n.p.	<0.1	<0.1	0.2	0.8	3.5	10.3	25.7
Other Volatiles	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	101.8	97.5	97.0	97.9	97.7	94.2	95.6	96.6
	97.3 ± 2.1							

* Results for additional radioactive fractions (M5, M12, M13, M14), individually not exceeding 1.3%, were not presented in detail by the author.

- Not detected

a Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%) detected in the application solution before and after application.

Table 95: Distribution and characterisation of radioactivity in the Mussig soil (soil II) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20±2ºC and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.* All values as % of applied radioactivity (AR).

	days after application							
	0	**1**	**3**	**7**	**14**	**28**	**56**	**120**
Parent	89.9	41.5	0.8	2.5	0.8	0.4	-	-
	90.4	32.0	3.3	2.1	-	0.4	-	-
Mean	90.1	36.7	2.1	2.3	0.4	0.4	-	-
Carbendazim	5.2	39.9	62.6	55.8	45.3	30.3	13.1	3.1
	4.6	44.5	63.0	59.1	44.7	28.2	13.0	3.3
Mean	4.9	42.2	62.8	57.4	45.0	29.2	13.1	3.2
DX-105	0.9	1.1	0.5	-	-	-	-	-
	0.6	1.2	0.7	-	-	-	-	-
Mean	0.7	1.2	0.6	-	-	-	-	-
2-AB	-	-	1.6	3.1	5.5	5.9	3.4	1.5
	-	-	1.3	2.3	6.7	4.2	3.9	1.3
Mean	-	-	1.5	2.7	6.1	5.0	3.7	1.4
CM-0237 (M2) [a]	-	2.9	5.6	8.0	4.4	5.0	3.2	2.7
	-	4.3	6.1	5.2	5.5	4.9	3.4	3.1
Mean	-	3.6	5.9	6.6	4.9	4.9	3.3	2.9
CM-0238 (M1) [a]	0.2	2.6	-	-	-	-	-	-
M3	-	-	2.8	-	2.3	1.3	1.3	0.6
M6	-	-	-	-	0.6	0.7	-	1.2
M7	-	0.7	3.9	4.6	4.0	2.7	2.8	2.5
M8	-	1.7	-	-	0.3	0.2	0.4	0.4
Extractable	98.7	86	69.9	57.7	48.1	32.3	11.8	3.4
Soxhlet	n.p.	6.7	11.7	17.2	17.3	13.9	16	11.9
Total extractable	98.7	92.7	81.6	74.9	65.4	46.2	27.8	15.3
Non-extractable	2.1	5.1	16.3	22.5	31.0	48.1	63.3	73.2
$^{14}CO_2$	n.p.	<0.1	0.2	0.5	0.9	1.5	3.7	7.6
Other Volatiles	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	100.7	97.8	98.1	97.9	97.4	95.8	94.8	96.1
	97.3 ± 1.8							

* Results for additional radioactive fractions (M5, M11, M12, M13), individually not exceeding 0.8%, were not presented in detail by the author.

- Not detected

a Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%) detected in the application solution before and after application.

Table 96: Distribution and characterisation of radioactivity in the Speyer 2.3 soil (soil III) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20 ± 2ºC and 46% MWHC. Mean of duplicates is given. For the parent and the metabolites that occurred in highest concentrations, results from the two individual replicates are also presented.* All values as % of applied radioactivity (AR).

	days after application							
	0	**1**	**3**	**7**	**14**	**28**	**56**	**120**
Parent	89.2	31.1	1.5	1.0	-	-	-	-
	90.3	29.0	1.6	-	-	-	-	-
Mean	89.7	30.0	1.6	0.5	-	-	-	-
Carbendazim	5.4	44.6	68.0	58.4	51.1	37.6	23.1	13.0
	5.9	45.4	64.3	58.3	51.6	39.1	23.2	11.7
Mean	5.7	45.0	66.1	58.4	51.4	38.3	23.1	12.3
DX-105	0.7	1.0	-	-	-	-	-	-
	0.6	0.9	-	-	-	-	-	-
Mean	0.6	1.0	-	-	-	-	-	-
2-AB	-	-	0.7	-	2.3	1.6	1.9	2.6
	-	-	0.7	-	2.2	1.8	1.8	-
Mean	-	-	0.7	-	2.3	1.7	1.8	1.3
CM-0237 (M2) [a]	-	5.4	11.2	7.2	7.9	6.6	3.3	2.3
	-	6.0	8.5	9.6	8.3	6.2	3.7	2.3
Mean	-	5.7	9.8	8.4	8.1	6.4	3.5	2.3
CM-0238 (M1) [a]	0.4	5.0	-	0.5	-	-	-	-
M3	-	-	2.1	0.9	1.4	1.0	0.8	0.4
M6	-	-	0.3	-	1.1	1.5	0.1	1.6
M7	0.2	1.1	2.0	2.2	2.1	1.5	1.4	0.8
M8	-	0.2	0.2	-	0.2	0.4	0.2	0.2
Extractable	99.4	86.3	73.3	58.5	50.8	36.8	20.7	5.1
Soxhlet	n.p.	6.5	11.8	16.4	17.7	15.9	15.6	18.2
Total extractable	99.4	92.8	85.1	74.9	68.5	52.7	36.3	23.3
Non-extractable	1.4	5.7	14.8	20.1	27.2	39.8	56.7	65.9
$^{14}CO_2$	n.p.	<0.1	0.1	0.4	0.7	1.4	3.4	7.3
Other Volatiles	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	100.8	98.5	100.1	95.4	96.4	93.9	96.4	96.5
	97.3 ± 2.6							

* Results for additional radioactive fractions (M5, M11, M12, M13, M15), individually not exceeding 1.9%, were not presented in detail by the author.

- Not detected

a Amounts of M1 and M2 were corrected for mean amounts of M1 (2.7%) and M2 (1.3%), which were detected in the application solution before and after application.

Table 97: Recovery of radioactivity as % AR in the different extraction steps, characterisation of the extractable radioactivity after harsh extraction and radioactivity in organic matter fractions, analysed in one sample taken at day 120.

	Bretagne Soil I	**Mussig Soil II**	**Speyer 2.3 Soil III**
Non-extractable after extraction at room temperature and Soxhlet extraction	40.3	72.5	63.8
Extractable radioactivity by "harsh extraction"	14.7	5.6	30.3
Carbendazim	7.2	1.1	9.0
2-AB	6.3	1.2	18.1
CM-0237 (M2)	0.8	1.9	0.6
CM-0238 (M1)	0.2	0.1	0.3
Others	0.2	1.3	2.3
Non-extractable after "harsh extraction"	25.6	66.9	33.5
Soluble fraction at low pH (fulvic acids)	10.1	8.3	7.7
Soluble fraction at high pH (humic acids)	7.5	1.1	11.7

	Bretagne Soil I	Mussig Soil II	Speyer 2.3 Soil III
Insoluble fraction (humins)	8.1	57.5	14.1

The author noted that carbendazim was often not retained on the HPLC column and eluting partially after about 5 minutes and after about 15 minutes (see one example in the figure below). The author explained the reduced retention by the low pH value in the samples (pH 5.5) that increased the polarity of carbendazim (pKa = 4.2 according to EFSA conclusion, 2010). The author added that an interaction with the organic solvent present in the sample caused lack of retention. This could be confirmed by partial evaporation of the organic solvent, which the author tested with one sample. The author further mentioned that the use of organic solvent was necessary to obtain complete recoveries. The author added up the two peaks and considered the sum as carbendazim.

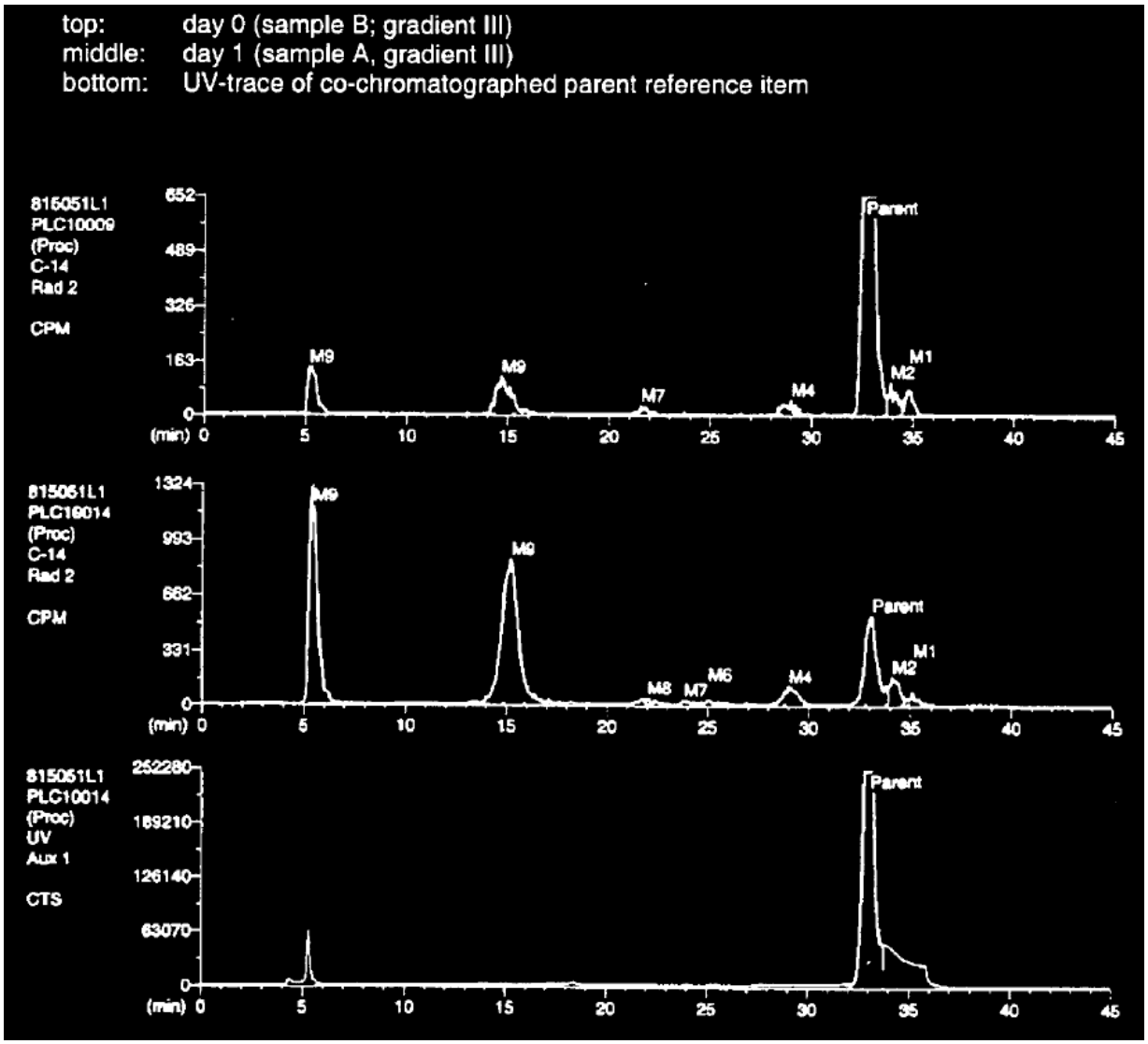
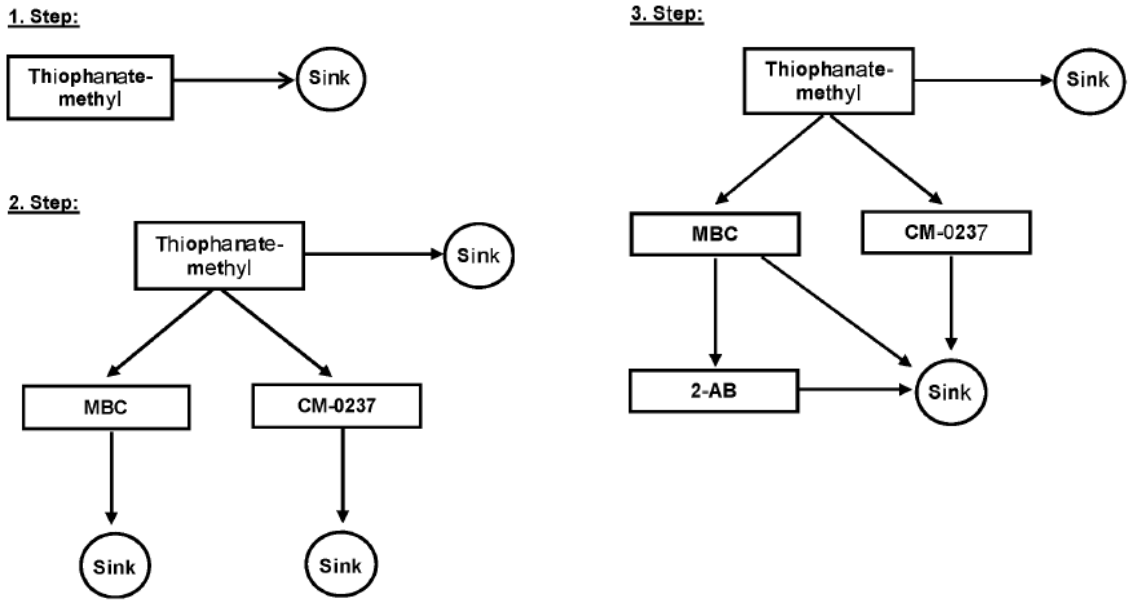


Figure 8.1.1.1-1. HPLC chromatograms of the soil extracts of soil I (Bretagne) on incubation day 0 (top) and day 1 (middle). M9 corresponds to carbendazim. Reference standard of parent (bottom). Figure is taken from Völkl (2002).

The degradation rates of thiophanate-methyl as well as the metabolites carbendazim and M2 were calculated by Völkl (2002). For the metabolites, decline fits were performed. DT_{50}s of thiophanate-methyl varied from 0.48 to 0.74 days. DT_{50}s of carbendazim ranged from 23.1 to 57.8 days and DT_{50}s of CM-0237 (M2) from 4.5 to 85.6 days.

The kinetic evaluation was superseded by the re-evaluation of Kiesel and Geibel (2016a,b), performed according to FOCUS guidance (2006, 2014). The statistical summaries of the kinetic analysis for thiophanate-methyl as well as the metabolites carbendazim (MBC), CM-0237 and 2-AB are presented in the table below. The degradation of thiophanate-methyl was best described by SFO in all three soils with DT_{50}s < 1 day (0.48–0.70 d). Additionally, pathway fits were performed that included parent and metabolites carbendazim, CM-0237 and 2-AB according to the below scheme (Evaluation Table Open point 4.3). During peer review, the applicant submitted an additional kinetic evaluation (Kiesel, Drechsler & Geibel, 2017a,b), to provide estimated parameters for the metabolite CM-0237 also for the Bretagne soil (Evaluation table, Data requirement 4.2). As a result of this revised pathway fit for the Bretagne soil, some minor changes were noted for the other metabolites. The below presentation of results have been amended accordingly.



The SFO-SFO results for the metabolites were considered as acceptable by the authors. CM-0237 was originally not included in the kinetic evaluation of the results for the Bretagne soil (I), presumably because the levels were <5% AR. For the metabolite 2-AB the χ2-%-errors were relatively high, in particular in the soil Speyer 2.3 (III). The visual fits were also not ideal. The RMS nevertheless accepted these results in the first draft RAR but during peer review (experts' consultation September 2017) it was decide to remove the results for 2-AB for the soil Speyer 2.3 from th data set. The estimated formation fractions for the metabolites are presented in table below. The visual assessments of the fits for parent and the metabolites carbendazim, CM-0237 and 2-AB are presented in the figures below.

Table 98: Summary of kinetic re-analysis by Kiesel and Geibel (2016a,b) of the data from Völkl (2002); for the Bretagne soil the final pathway fit was done by Kiesel, Drechsler & Geibel (2017a,b). For thiophanate-methyl, only results for parent-only fit are shown. For the metabolites, the results from pathway-fit with thiophanate-methyl and the major metabolites (carbendazim, CM-0237 and 2-AB) are given.

Substance	Soil	Kinetic model	Mo	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Thiophanate-methyl	Bretagne (soil I)	**SFO**	96.34	k=1.5826	5.0	1.4E-08	1.4	1.8	0.44	1.5
		FOMC	96.34	α=5.584 β=3.034	5.2	n.r.	-9.4 -6.4	20.6 12.5	- [a]	- [a]
	Mussig (soil II)	**SFO**	96.22	k=0.9936	5.8	7.0E-11	0.9	1.1	0.70	2.3
		FOMC	96.22	α=1.25E+5 β=1.26E+5	6.3	n.r.	1.2E+5 1.2E+5	1.3E+5 1.3E+5	0.70	2.3
	Speyer 2.3 (soil III)	**SFO**	96.77	k=1.1812	1.9	1.9E-10	1.1	1.2	0.59	1.9
		FOMC	96.77	α=2.55E+5 β=2.16E+5	2.2	n.r.	2.5E+5 2.1E+5	2.6E+5 2.2E+5	0.59	1.9
Carbendazim	Bretagne (soil I)	SFO-SFO	0	k=0.01096	4.7	2.0E-16	9.3E-3	0.013	63.2	210
	Mussig (soil II)	SFO-SFO	0	k=0.03144	2.1	<2E-16	0.029	0.034	22.0	73.2
	Speyer 2.3 (soil III)	SFO-SFO	0	k=0.01843	5.0	<2E-16	0.016	0.021	37.6	125
CM-0237	Bretagne (soil I)	SFO-SFO	0	k=0.2438	30.2	2.7E-05	0.14	0.35	2.8	9.4
	Mussig (soil II)	SFO-SFO	0	k=0.00801	8.5	2.8E-05	0.004	0.01	86.5	287
	Speyer 2.3 (soil III)	SFO-SFO	0	k=0.01491	7.7	1.2E-08	0.010	0.019	46.5	154
2-AB	Bretagne (soil I)	SFO-SFO [b]	0	k=0.05135	20.0	1.6E-05	0.03	0.07	13.5	44.8
	Mussig (soil II)	**SFO-SFO** [b]	0	k=0.06027	16.1	1.3E-07	0.04	0.08	11.5	38.2
	Speyer 2.3 (soil III)	SFO-SFO [b]	0	k=0.02961	33.6	9.7E-3	0.0056	0.054	- [c]	- [c]

n.r Not relevant.

n.p Not provided.

a Author considered the results statistically not reliable.

b Carbendazim as pre-cursor.

c Result considered as not reliable at experts' consultation (September, 2017).

Table 99: Formation fractions of carbendazim, CM-0237 and 2-AB, according to kinetic re-analysis by Kiesel and Geibel (2016a,b) of the data from Völkl (2002); for the Bretagne soil the final pathway fit was done by Kiesel, Drechsler & Geibel (2017a,b).

Formation	Soil	Formation fraction	St. dev.
Thiophanate-methyl → Carbendazim	Bretagne (soil I)	0.79	0.02
	Mussig (soil II)	0.72	0.02
	Speyer 2.3 (soil III)	0.69	0.015
Thiophanate-methyl → CM-0237	Bretagne (soil I)	0.055	0.01
	Mussig (soil II)	0.064	0.007
	Speyer 2.3 (soil III)	0.099	0.008
Carbendazim → 2-AB	Bretagne (soil I)	0.36	0.07
	Mussig (soil II)	0.33	0.04
	Speyer 2.3 (soil III)	0.12 [a]	0.03 [a]

a Result considered as not reliable at experts' consultation (September, 2017).

Table 100: Summary of modelling endpoints for thiophanate-methyl and its metabolites carbendazim, CM-0237 and 2-AB, as accepted by the RMS.

Substance	Soil	Modelled value		Normalised (pF 2, 20°C) SFO DT_{50} [days] for modelling	
		Kinetic model	DT_{50}[days]	Correction factor [a]	Norm. DT_{50}
Thiophanate-methyl	Bretagne (silt loam)	SFO	0.44	1.00	0.44
	Mussig (clay loam)	SFO	0.70	1.00	0.70
	Speyer 2.3 (sandy loam)	SFO	0.59	0.944	0.56
Carbendazim	Bretagne (silt loam)	SFO-SFO	63.2	1.00	63.2
	Mussig (clay loam)	SFO-SFO	22.0	1.00	22.0
	Speyer 2.3 (sandy loam)	SFO-SFO	37.6	0.944	35.5
CM-0237	Bretagne (silt loam)	SFO-SFO	2.8	1.00	2.8
	Mussig (clay loam)	SFO-SFO	86.5	1.00	86.5
	Speyer 2.3 (sandy loam)	SFO-SFO	46.5	0.944	43.9
2-AB	Bretagne (silt loam)	SFO-SFO	13.5	1.00	13.5
	Mussig (clay loam)	SFO-SFO	11.5	1.00	11.5
	~~Speyer 2.3 (sandy loam)~~	~~SFO-SFO~~	~~23.4~~	~~0.944~~	~~22.1~~

n.a. Not available.

a Moisture correction factor calculated according to FOCUS (2000): fmoisture = (Θ/Θref)$^{0.7}$. Moisture at 46% MWHC as provided in the study was used for the calculation together with the default moisture content at pF 2 (field capacity). No normalisation for temperature necessary as study was performed at a temperature of 20ºC.

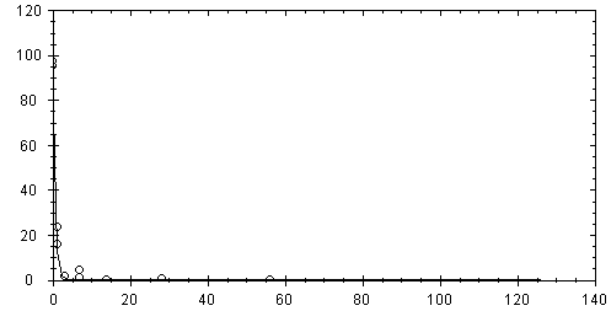
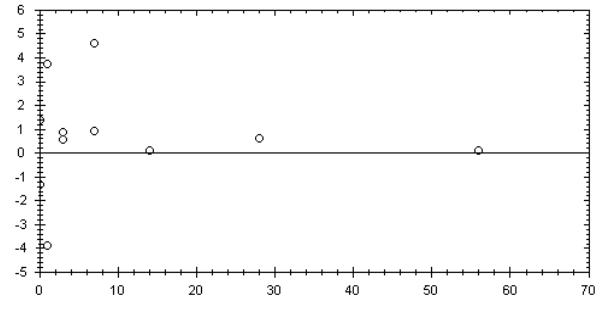
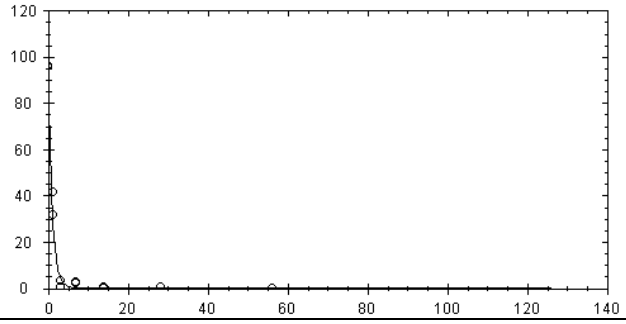
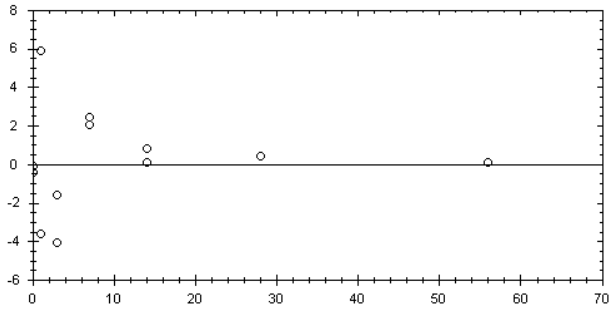
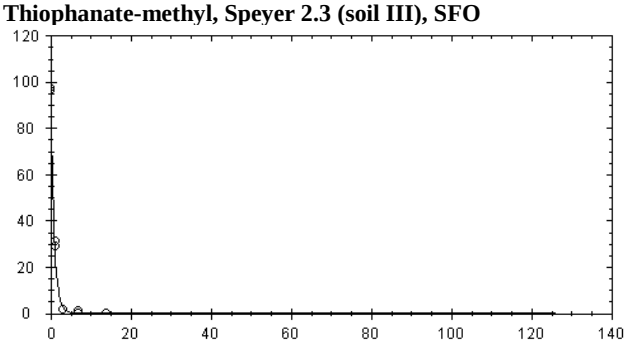
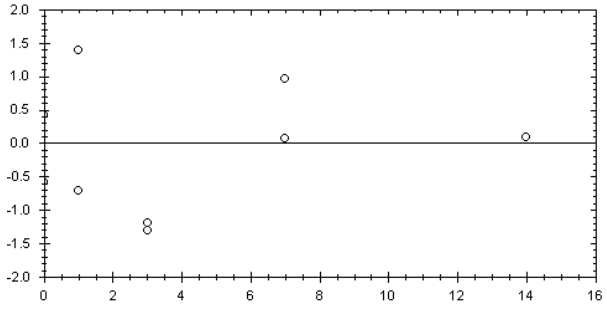
Measured & Predicted vs. Time	Residual Plot
Thiophanate-methyl, Bretagne (soil I), SFO	
	
Thiophanate-methyl, Mussig (soil II), SFO	
	
Thiophanate-methyl, Speyer 2.3 (soil III), SFO	
	

Figure 8.1.1.1-2. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent-only), from Kiesel and Geibel (2016b). Only the best-fit model for each soil is shown.

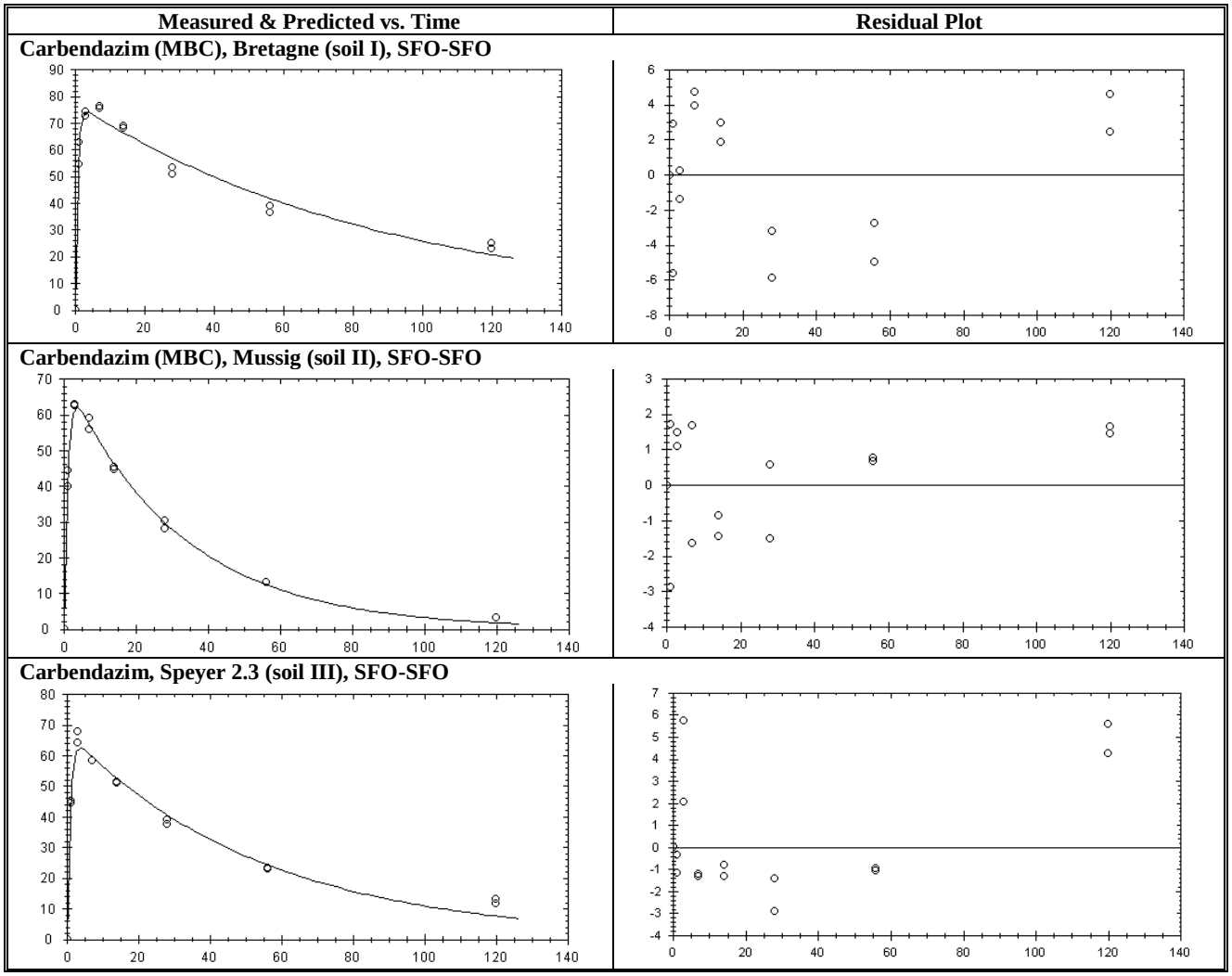


Figure 8.1.1.1-3. Visual fit and plot of residuals for kinetic fitting of the primary metabolite carbendazim (MBC; pathway-fit), from Kiesel and Geibel (2016b).

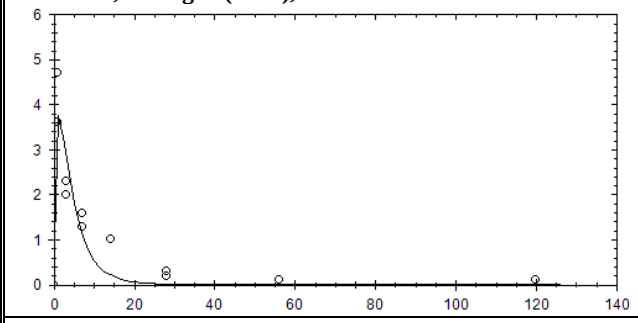
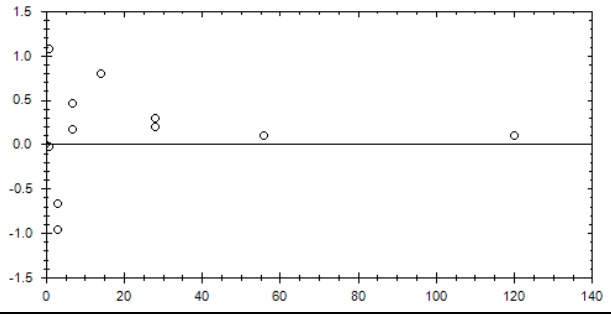
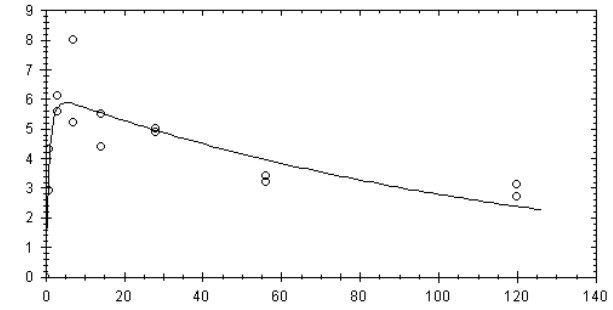
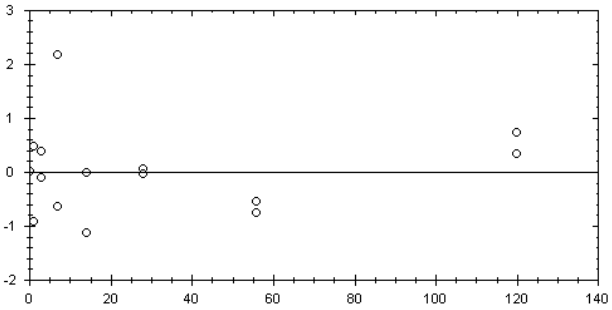
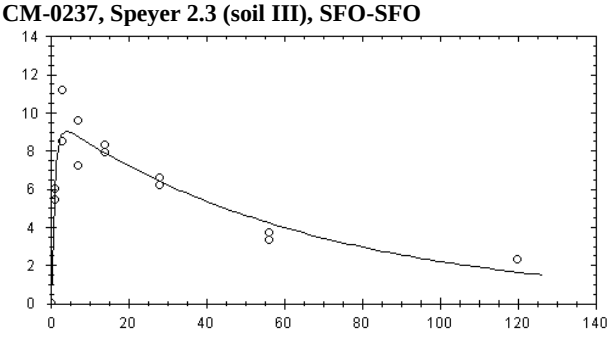
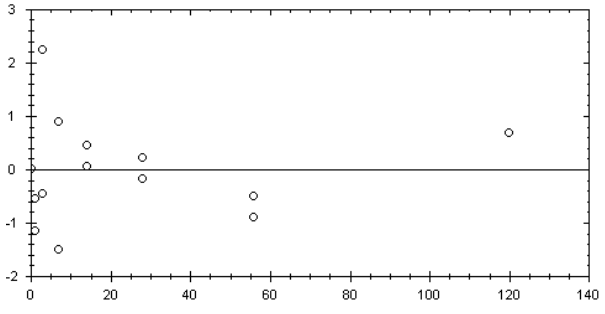
Measured & Predicted vs. Time	Residual Plot
CM-0237, Bretagne (soil I), SFO-SFO	
	
CM-0237, Mussig (soil II), SFO-SFO	
	
CM-0237, Speyer 2.3 (soil III), SFO-SFO	
	

Figure 8.1.1.1-4. Visual fit and plot of residuals for kinetic fitting of the primary metabolite CM-0237 (pathway-fit), from Kiesel and Geibel (2016b). Graphs for the Bretagne soil are from Kiesel, Drechsler & Geibel (2017b).

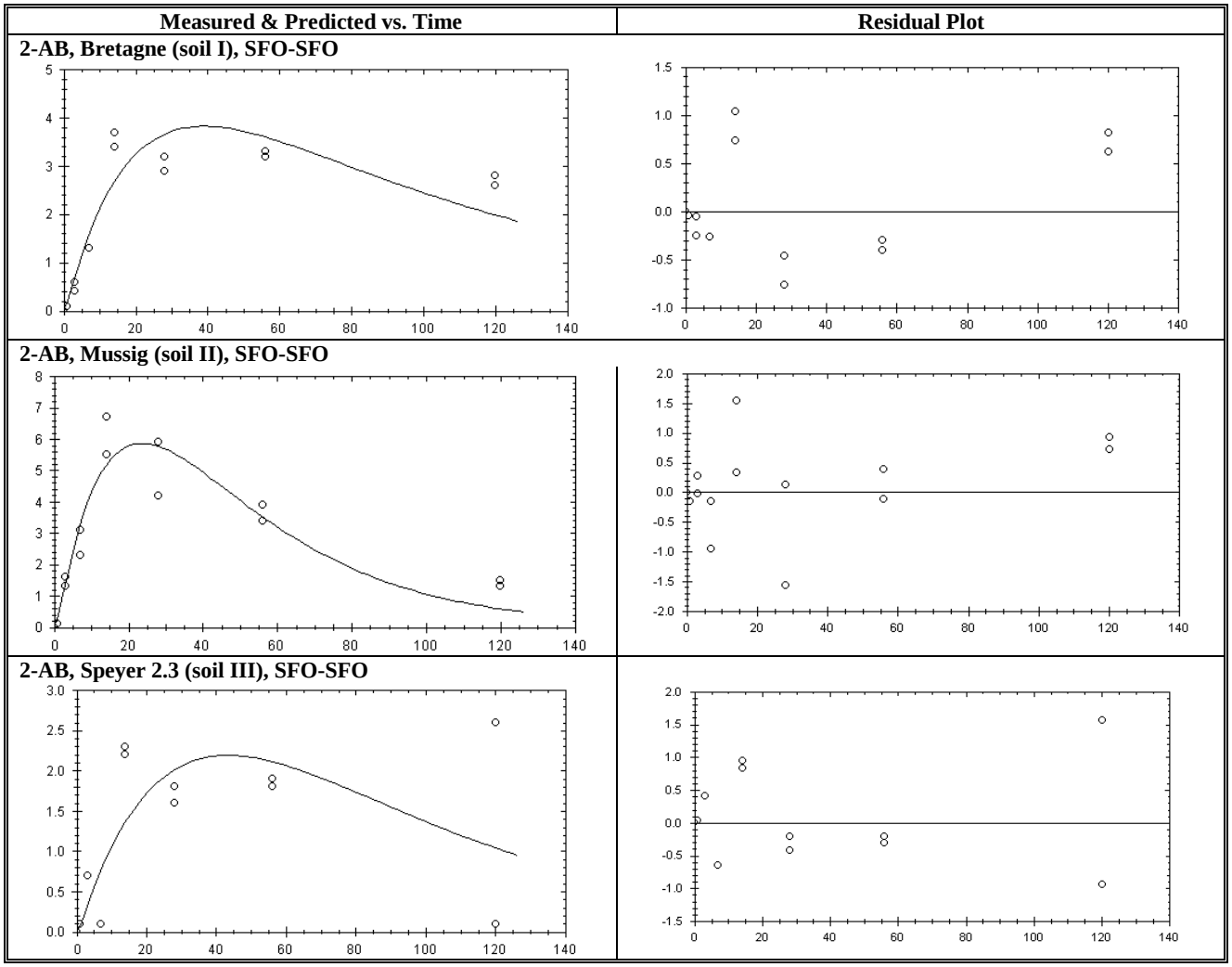


Figure 8.1.1.1-5. Visual fit and plot of residuals for kinetic fitting of the secondary metabolite 2-AB. Results from the pathway-fit with carbendazim as precursor, taken from Kiesel and Geibel (2016b).

RMS comments and conclusions

The study by Völkl (2002) was evaluated and accepted in the Addendum (2003) to the DAR (1997), and is also accepted for the purpose of renewal. The study was well performed and reported. The RMS agrees with the proposed degradation pathway and concludes that thiophanate-methyl was rapidly degraded into carbendazim (MBC) and CM-0237 (M2). Carbendazim was likely to be formed via the (minor) metabolite DX-105 and degraded into metabolite 2-AB. The RMS suggests to consider 2-AB further for exposure and risk assessment in accordance with current data requirements (Regulation (EU) No 283/2013). The applicant did not agree to this since one of the measurements was not above 5.0% AR. During peer review it was decided to include 2-AB in the residue definition for soil, groundwater and surface water (Evaluation table Open point 4.1).

The RMS considered it difficult to judge whether the radioactivity eluting after approximately 5 minutes (see text and figure above) corresponded (exclusively) to carbendazim or to another (unknown) metabolite. In the latter case, a potential additional major metabolite might have been missed. However, most of the provided HPLC chromatographs indicated that the first peak was minor compared to the second peak of carbendazim or that there was no peak after 5

minutes. But as there were no HPLC chromatograms for all samples and as at least one of the figures presented indicated that the first peak was larger than the second peak (for day 1, sample B), some uncertainty remains. The RMS assumes that it will not be possible to characterise the radioactivity in those first eluting peaks any longer, as the study is almost 15 years old. Therefore, the RMS suggests to accept the approach the author had chosen by adding up the two peaks to carbendazim and use the data for the risk assessment. At least for carbendazim, this approach can be considered conservative.

The re-evaluation of the rate of degradation of the metabolites by Kiesel and Geibel (2016a,b), and for the Bretagne soil by Kiesel, Drechsler & Geibel (2017a,b), was performed according to the FOCUS guidance and is considered acceptable. The RMS agrees with the authors' choice of SFO as best-fit model for parent, and SFO-SFO for the metabolites. As a general principle, the RMS also accepts χ2-%-error above 15% since this level should not be considered as a definitive criterion.

Reference:	**1. Adam, D. (2014a)** ^{14}C-Thiophanate-methyl: Route and Rate of Degradation in One Soil under Aerobic Conditions. **2. Adam, D. (2016)** ^{14}C-Thiophanate-methyl - Route and Rate of Degradation in One Soil under Aerobic Conditions
Report No.:	1. RD-02985 2. RD-03848
Document No.:	1. 722-001 2. 781-005
Guideline:	OECD TG No. 307 (2002)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal.
Material and methods:	
Test material:	Phenyl-UL-^{14}C-labelled thiophanate-methyl
Lot/Batch No:	XIX/17/1
Purity:	97.7% (determined by HPLC before treatment; 97.2% after treatment)
Test concentration:	1.42 mg/kg, corresponding to 1.1 kg a.s./ha (assuming a distribution of the test item in the top 5 cm soil layer and a bulk density of 1.5 g/cm^3). ^{14}C-thiophanate-methyl was dissolved in acetonitrile (≤1%), but the solvent was not evaporated due to the fast degradation of the test item.
Test system:	A new study was carried out to fulfil the requirements of determining a forth degradation rate for thiophanate-methyl. 100 g dry weight soil were filled into all-glass metabolism flasks in a moist air flow-through systems with absorption traps with ethylene glycol and 2N NaOH for organic volatiles and $^{14}CO_2$, respectively. Presence of $^{14}CO_2$ in the trapping solution was confirmed by $BaCO_3$-precipitation in selected samples. (Figure 1 of the report indicated that an additional trap containing 1 M H_2SO_4 was used. During the evaluation the applicant submitted a statement (Adam, 2016) in which the study author clarified that Figure 1 was erroneous; no such trap was used.)
Test conditions:	Soils were sieved (2 mm), acclimated at 20ºC for 5 days and incubated in the dark at 20.9±0.1ºC and at a moisture content of pF 2 under aerobic conditions.
Sampling time points:	Duplicate samples were taken for extraction and analysis after 0, 1, 3, 7, 14, 28, 55, 90, and 120 days. No sampling of volatiles on day 0.
Method of analysis:	All samples were extracted with acetonitrile:water (4:1; v/v) up to four times and

additionally with a Soxhlet extraction using acetonitrile:water (4:1; v/v) for four hours. Samples from day 120 were subject to an additional harsh extraction with acetonitrile:0.1M hydrochloric acid (4:1; v/v) for four hours under reflux conditions followed by an organic matter fractionation.

Radioactivity of individual extracts as well as radioactivity in the traps was analysed by LSC. Non-extractable radioactivity was analysed by LSC after combustion. Parent and metabolites were characterised with HPLC and/or TLC by co-chromatographic profiling of the pooled extracts and comparison of retention times. The following reference substances were used for metabolite identification: MBC (carbendazim), DX-105, FH-432, CM-0237, AV-1951, 2-AB, CM-0238.

The LOQ of the HPLC was 0.004% AR.

Kinetic analysis: A pathway fit was performed for thiophanate-methyl and the primary metabolite carbendazim (MBC) with the software tool CAKE v. 1.4 following FOCUS GD (2006, 2014). Only SFO-kinetics were presented and used to determine DT_{50} and DT_{90} for the two substances and the formation fraction of the metabolite.

Table 101: Soil characteristics.

		Speyer 5M
Origin		Germany
Soil plot history		No pesticides used for at least four years prior to the sampling.
Collection and storage		Collected in February 2013. Sieved (2 mm). Stored for about 1.5 months at 4°C. Acclimation at 20°C for about 5 days before study.
Soil type (USDA)		Sandy loam
Clay <2 µm	[%]	11.1
Silt 2–50 µm	[%]	29.7
Sand >50 µm	[%]	59.2
Water content at pF 2.0	[g/100 g soil]	23.0
pH ($CaCl_2$) / pH (H_2O)		7.9 / 7.3
Organic carbon	[g/100 g soil]	1.0
CEC	[mmol/100 g soil]	16.6
Biomass – start of incubation	[mg/100 g soil]	33.7
Biomass – end of incubation	[mg/100 g soil]	38.6

Results

The total recovery of individual samples ranged between 92.8 and 107.8% AR. The fraction of extractable radioactivity decreased from 91.4% AR at day 0 to 15.9% AR after 120 days, while the fraction of non-extractables increased from 7.8% AR at day 0 to 72.1% AR by day 120. The samples taken at day 1 deviated from the trend; the fraction of total extractable from samples taken at day 1 was lower than any sample taken up to day 14 and similarly the fraction of non-extractable was higher at day 1 than at day 14. Mineralisation to $^{14}CO_2$ reached a maximum of 7.6% AR at study termination. Other volatiles were below 0.1% AR throughout the study period.

Thiophanate-methyl decreased from 82.9% AR at day 0 to 0.3% AR after 55 days and was not detected thereafter. The concentration of the major metabolite carbendazim peaked at 48.3% AR after 3 days and decreased afterwards to 7.1% AR at the end of the study period. None of the other 19 observed metabolites exceeded 10% once or 5% at two consecutive sampling intervals. An unknown metabolite (M8) was once detected at a concentration of 6.8% AR (day 3) but was below 3% AR at the closest sampling intervals. Neither CM-0237 nor 2-AB exceeded 4.5% AR on average at individual sampling occasions.

Samples taken after 14 and 28 days showed quite a spread between the replicates both for the metabolites carbendazim, CM-0237, 2-AB and the parent. The RMS considers that this suggests some problems with the analysis for those sampling intervals.

The samples taken after 120 days were subject to an additional harsh extraction and the remaining non-extractable radioactivity subject to organic matter fractionation. Of the 64.4% AR that remained non-extractable after the harsh extraction, 56% were bound to the humin fraction (i.e. 36.1% AR), and roughly 20% to fulvic and humic acids, respectively, corresponding to 14% AR.

Adam (2014a) proposed the same route of degradation as Völkl (2002), see Figure 8.1.1.1-6.

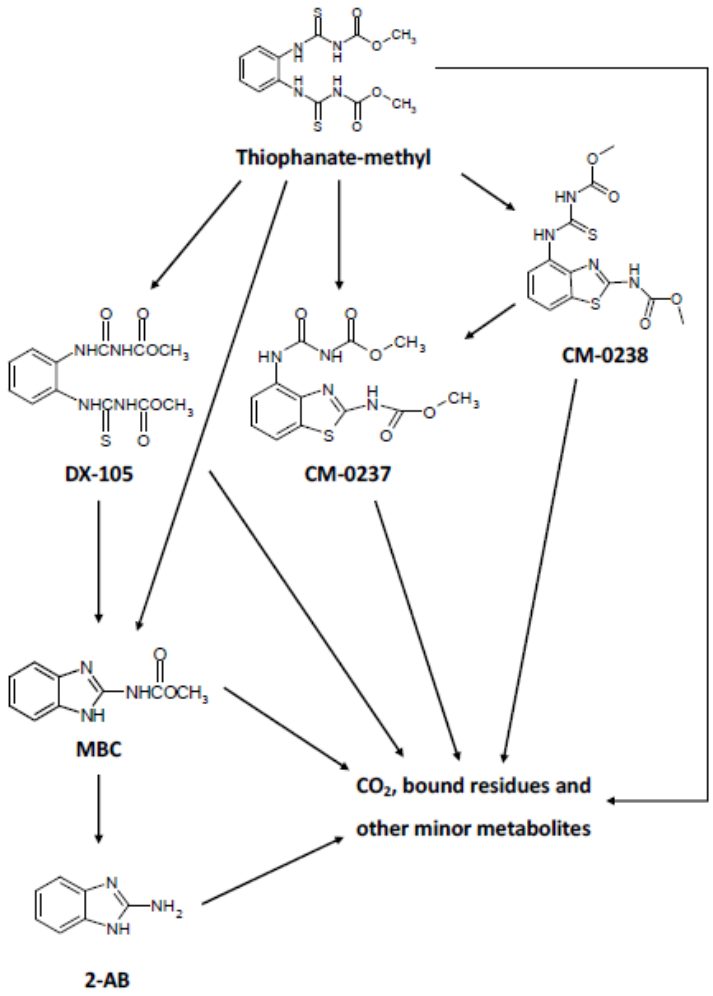


Figure 8.1.1.1-6. Degradation pathway in aerobic soils, as proposed by Adam (2014).

Table 102: Distribution and characterisation of radioactivity in the Speyer 5M soil (sandy loam) treated with phenyl-14C-labelled thiophanate-methyl and incubated at 20.9±0.1°C and at pF 2. Mean of duplicates is given. For the parent and the metabolites carbendazim, 2-AB and CM-0237, results from the two individual replicates are also presented. All values are given as % of applied radioactivity (AR).

	days after application								
	0	**1**	**3**	**7**	**14**	**28**	**55**	**90**	**120**
Parent	81.7	6.9	2.1	2.3	0.7	2.6	0.7	-	-
	84.0	7.6	1.7	3.1	-	0.4	-	-	-
Mean	82.9	7.3	1.9	2.7	0.4	1.5	0.3	-	-
Carbendazim	3.2	41.1	48.9	43.1	34.8	42.7	16.2	9.7	5.8
	3.1	39.2	47.7	43.7	43.9	29.4	13.3	8.5	8.3
Mean	3.2	40.1	48.3	43.4	39.3	36.0	14.8	9.1	7.1
CM-0237	-	2.9	-	3.6	3.1	3.9	4.2	2.7	2.0
	-	3.0	0.4	3.3	5.4	5.0	4.3	2.8	2.1
Mean	-	3.0	0.2	3.4	4.2	4.4	4.2	2.8	2.0
2-AB	-	-	-	2.8	4.6	4.6	2.4	0.7	0.6
	-	-	-	2.8	3.1	1.5	2.0	0.7	1.6
Mean	-	-	-	2.8	3.8	3.0	2.2	0.7	1.1
DX-105	0.8	1.7	1.3	1.0	0.9	0.6	0.3	0.3	0.1
CM-0238	-	1.1	0.8	0.5	0.1	0.1	-	0.2	-
AV-1951	-	-	-	-	0.5	0.9	0.6	0.3	0.3
M8	0.8	1.6	6.8	2.8	2.5	2.0	2.2	1.8	1.3
M10	-	3.1	3.0	3.2	3.1	0.7	1.1	0.9	0.7
M11	-	0.7	2.2	-	-	-	-	-	-
M15	3.7	0.6	1.1	1.6	2.0	2.2	0.4	3.0	0.2
Other metabolites [a]	-	0.7	5.0	4.9	5.2	5.2	3.5	3.8	3.0
Extractable	85.3	47.5	45.7	42.9	44.3	41.9	17.4	10.2	9.8
Soxhlet	6.1	12.6	24.9	23.4	17.9	14.8	12.3	12.6	6.1
Total extractable	91.4	60.0	70.6	66.3	62.2	56.7	29.7	22.8	15.9
Non-extractable	7.8	39.4	28.8	32.9	34.7	41.3	64.7	70.9	72.1
$^{14}CO_2$	n.p.	<0.1	0.2	0.4	0.9	1.8	5.7	7.1	7.6
Other Volatiles	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	99.1	99.4	99.6	99.6	97.7	99.7	100.0	100.8	95.6
MEAN ± SD	**99.1 ± 3.1**								

- Not detected.

n.p. Not performed.

SD Standard deviation.

a Sum of other minor metabolites identified. None exceeded individually 2% AR.

Table 103: Radioactivity recovery as % AR in the different extraction steps and in organic matter fractions, analysed at day 120.

	Speyer 5M soil (sandy loam)
Non-extractable after extraction at room temperature and Soxhlet extraction	72.1
Extractable radioactivity by “harsh extraction”	7.7
Non-extractable after “harsh extraction”	64.4
Soluble fraction at low pH (fulvic acids)	13.9
Soluble fraction at high pH (humic acids)	14.4
Insoluble fraction (humins)	36.1

The kinetic evaluation was performed with the software package CAKE with a pathway-fit including thiophanate-methyl and the primary metabolite carbendazim (MBC). The results are presented in the tables and the figure below. No

results from parent-only simulations were provided and only SFO-results for parent and the metabolite presented. Additionally, day zero residues of metabolites were not added to the parent compartment and samples with concentrations <LOD were treated as missing values.

Therefore, the RMS performed new optimisations with the software KinGUI v.2.1, which are presented in tables and figure further below. No reliable results were obtained for thiophanate-methyl with the FOMC model. A pathway fit including CM-0237 could not be derived either. But a pathway fit with parent degrading to the primary metabolite carbendazim and the secondary metabolite 2-AB could be fitted. For 2-AB the χ2-%-error was relatively high and the fit did not capture the maximum observed. Nevertheless, the RMS suggests that the fit was good enough and the RMS suggests the estimates from the RMS' optimisation should be used for exposure assessment. The results for thiophanate-methyl and carbendazim were similar to those obtained by Adam (2014a).

Table 104: Summary of kinetic analysis by Adam (2014a). Only results from the pathway-fit with all SFO are shown.

Substance	Soil	Kinetic model	Mo [a]	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Thiophanate-methyl	Speyer 5M	SFO	82.8	k=2.3968	9.0	1.2E-18	2.1	2.7	0.29	0.96
Carbendazim	Speyer 5M	SFO-SFO	3.2	k=0.01727	8.5	2.1E-10	0.01	0.02	40.1	133

a The author did neither add the initial concentration of carbendazim (and other metabolites) to the concentration of the parent nor did they fix M0 for the metabolite to 0.

Table 105: Formation fraction for carbendazim, according to kinetic analysis by Adam (2014a).

Formation	Soil	Formation fraction	St. dev.
Thiophanate-methyl → Carbendazim	Speyer 5M	0.59	0.024

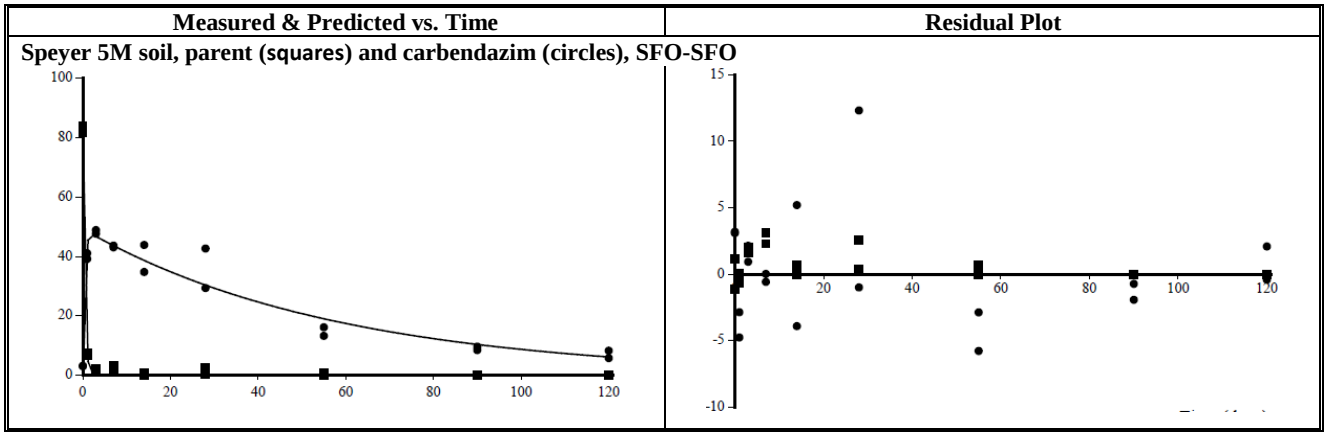


Figure 8.1.1.1-7. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent) and carbendazim. Results of the pathway-fit, from Adam (2014a).

Table 106: Summary of kinetic re-analysis by RMS based on data presented in Adam (2014a).

Substance	Soil	Kinetic model	Mo [a]	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Thiophanate-methyl	Speyer 5M	SFO (parent only)	91.4	k=2.5294	7.8	1.9E-10	n.a.	n.a.	0.27	0.91
		FOMC (parent only)	91.4	α=0.77566 β=0.03779	4.5	n.r.	0.5 0.02	1.1 0.09	- [a]	- [a]
Carbendazim	Speyer 5M	SFO-SFO	0	k=0.01728	9.1	1.3E-10	n.a.	n.a.	40.1	133
2-AB	Speyer 5M	SFO-SFO	0	k=0.13184	24.2	0.013	n.a.	n.a.	5.3	17.5

n.a. Not available.

n.r. Not relevant.

a Statistically not reliable.

Table 107: Formation fractions of the metabolites carbendazim and 2-AB, according to the kinetic analysis by the RMS, based on data from Adam (2014a).

Formation	Soil	Formation fraction	St. dev.
Thiophanate-methyl → Carbendazim	Speyer 5M	0.53	0.023
Carbendazim → 2-AB	Speyer 5M	0.75	0.30

Table 108: Summary of modelling endpoints for thiophanate-methyl and its metabolites carbendazim and 2-AB, as proposed by the RMS.

Substance	Soil	Modelled value		Normalised (pF 2, 20°C) SFO DT_{50} [days] for modelling	
		Kinetic model	DT_{50}[days]	Correction factor [a]	Norm. DT_{50}
Thiophanate-methyl	Speyer 5M	SFO	0.27	1.089	0.29
Carbendazim	Speyer 5M	SFO	40.1	1.089	43.6
2-AB	Speyer 5M	SFO	5.3	1.089	5.8

a Moisture correction factor calculated according to FOCUS (2000): ftemp = $Q10^{[(Tstudy - Tref)/10]}$ with Q10 = 2.58, Tstudy = 20.9°C and Tref = 20°C. No normalisation for moisture necessary as study was performed at pF 2.

Measured & Predicted vs. Time	Residual Plot
Thiophanate-methyl (SFO)	
Carbendazim (SFO-SFO)	
2-AB (SFO-SFO; precursor Carbendazim)	

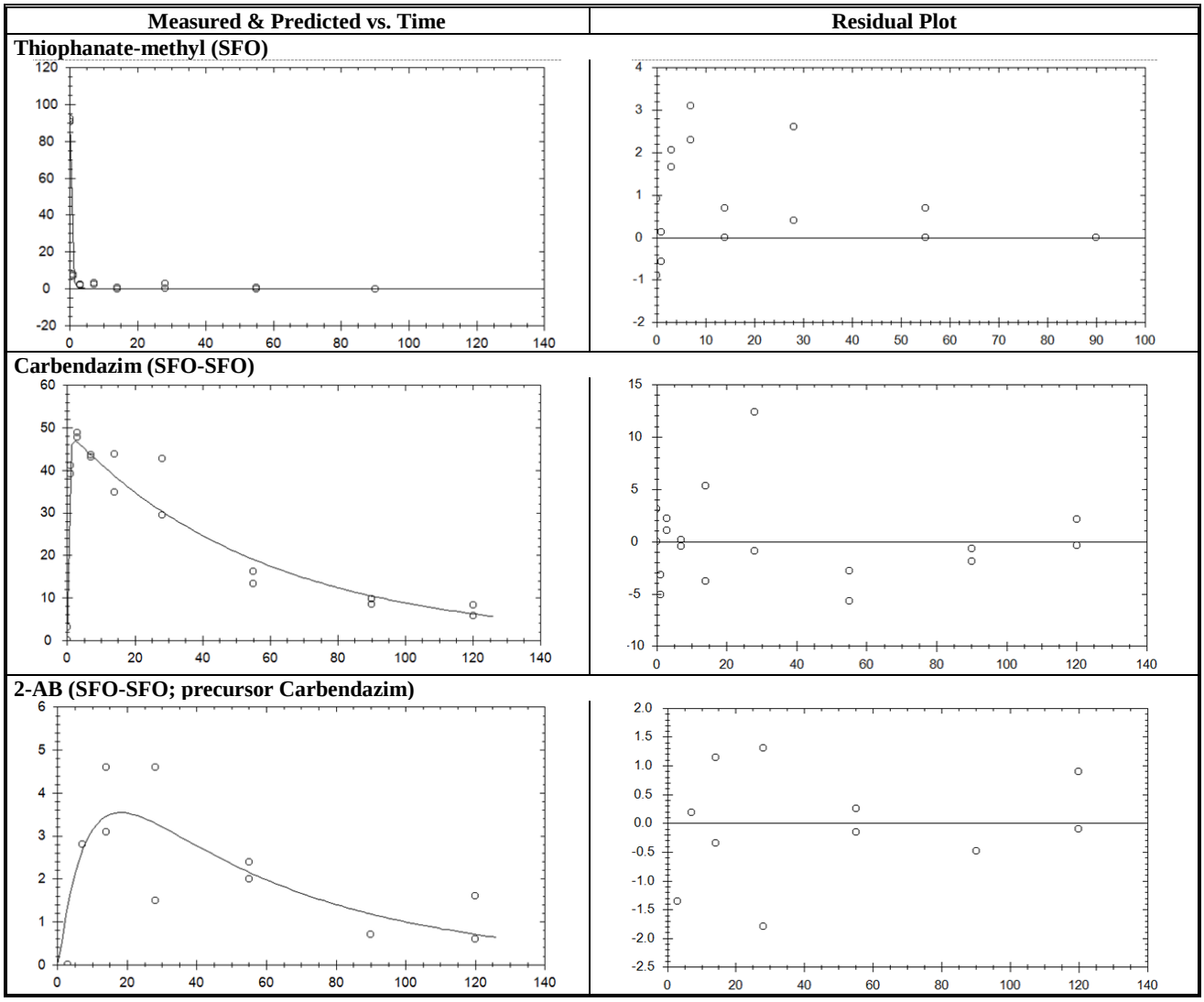


Figure 8.1.1.1-7. Visual fit and plot of residuals for kinetic fitting of thiophanate-methyl (parent), carbendazim and 2-AB. Results of the pathway-fit as re-calculated by the RMS, based on data from Adam (2014a).

RMS comments and conclusions

The laboratory study was performed according to the OECD TG No. 307 and the results well reported. The study is considered acceptable for the purpose of renewal of the approval of thiophanate-methyl. The kinetic evaluation of the data is not considered fully acceptable due to handling of day 0 values and results <LOD. Nevertheless, there were a few issues that render some uncertainty to the study results. The amount of total extractable and non-extractable radioactivity in samples taken at day 1 suggested some degree of uncertainty as the amount of extractable is higher thereafter and the amount of non-extractable lower (until day 14), respectively. Furthermore, especially the measurements of carbendazim at day 14 and 28 indicated uncertainty in the analyses.

The author did not discuss whether carbendazim was less retained on the HPLC column or might have potentially eluted in several peaks, as it was observed by Völkl (2002); see figure below. The author treated the first peak eluting after roughly four-five minutes as a separate, minor metabolite. The RMS agrees with this.

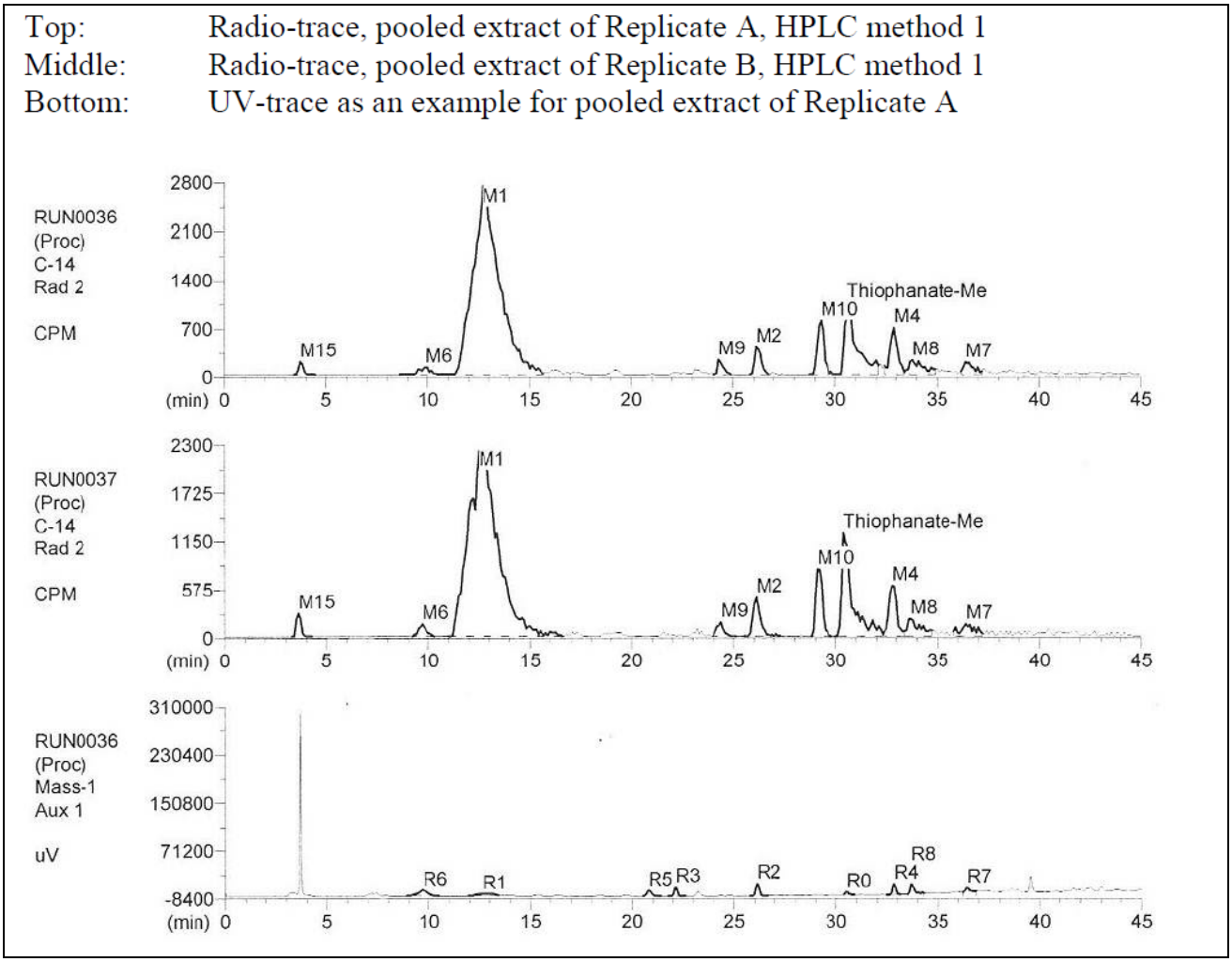


Figure 8.1.1.1-8. HPLC chromatograms of day 1 soil samples. Figure taken from Adam (2014). The reference items are denoted as follows: R1 = Carbendazim, R2 = DX-105, R3 = FH-432, R4 = CM-0237, R5 = AV-1951, R6 = 2-AB, R7 = CM-0238.

The author noted that "Thiophanate-methyl is known to hydrolyse extremely fast. Therefore, extracts were subjected to HPLC with a high concentration of organic solvent. This procedure resulted in an asymmetric peak-shape but had the advantage that only a small amount of Thiophanate-methyl hydrolysed. Hydrolysis during work-up for HPLC could be confirmed by TLC results. However, organic solvent in the sample did not interfere with spot-shape in TLC and therefore, a symmetric test item spot was observed". A very fast hydrolysis rate is in contrast to the results obtained in the hydrolysis study (with pure water), but hydrolysis rate may be faster in dissolved organic fractions. As it further seems that transformation due to the organic solvent can be excluded, the RMS accepted the procedure chosen by the author to analyse the results.

As the kinetic evaluation by the author did not entirely follow FOCUS degradation kinetics guidance (2006, 2014), the results from the re-evaluations performed by the RMS are considered more appropriate. The results obtained by the RMS for thiophanate-methyl and carbendazim were very similar to the results obtained by the author and provided reliable endpoints. The RMS additionally obtained DT_{50} and formation fraction (with carbendazim as precursor) for the secondary metabolite 2-AB. Despite a χ2-%-error >15, the RMS considered the fit acceptable based on the visual and

statistical (p-value) inspection. This is in line with FOCUS degradation kinetics report (2006, 2014) that indicates that a χ2-%-error of 15% should not be considered a cut-off but rather a guidance value.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

Reference:	**1. Otto, S. (1975)** Verhalten des Pflanzenschutzmittel-Wirkstoffes im Boden **2. Zillgens, B. (2007a)** Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model MakerTM 4.0
Report No.:	1 A22991, BOD95-00442 2. DuPont-21656
Guideline:	BBA leaflet no. 36 (February 1973)
GLP:	1. No
Validity	1. Valid

Material and methods:

An aerobic soil degradation study was conducted according to BBA leaflet no. 36. Benzim-idazol-2-carbamid-säure-methylester (BCM = Carbendazim) was added to two standard soils characterized in the table below.
Each soils was treated with 3 ppm BCM. The amount of applied active substance is about 1.5-fold higher than the maximum application rate of 2 x 250 g as/ha All soil samples were adjusted to 40 % maximum water holding capacity (MWHC) and were incubated in darkness at 22°C up to 380 – 480 days.
Samples were taken at time intervals 0, 20, 40, 55, 85, 240 and 380 days (sand) and 0, 10, 25, 75, 120, 240, 380 and 480 days (loamy sand) for subsequent analysis.
The samples were extracted with 10 % ethyl acetate in methanol. For the quantification of BCM in the residues of sand soil a detection of ^{14}C-radioactivity was used followed by radio thin layer chromatography. The detection limit was 0.01 ppm. For the quantification of BCM in the residues of loamy sand soil a UV-spectroscopy was carried out. Detection limit was 0.05 ppm.

Kinetic analyses: DT_{50}/DT_{90} were determined graphically in the original report (Otto, 1975). DT_{50}/DT_{90} were re-calculated in accordance with FOCUS guidance (in Zillgens, 2007a) and those results were the final ones.

Table 109: Soil properties.

Soil property	Standard soil 1	Standard soil 2
Particle size distribution (DIN) (%)		
< 2 µm	10.1	19.5
Classification (BBA)	sand	loamy sand
Organic carbon (%)	2.58	1.0
pH ($CaCl_2$)	6.8	5.2
Maximum water holding capacity (w/w %)	n.a.	n.a.

Results

The decline of active substance in both soils during the incubation period is summarised in the below table. A mass balance was not completed for the various incubation periods used in this study. Volatilisation was not measured. At the end of the study a recovery rate of 88% and 81% for standard soil 1 and standard soil 2 was determined. In the soil extracts primarily the active substance is detected by radio thin layer chromatography. Extractable degradation products were only formed in traces. The main part of degradation products were probably bound in form of non-extractable residues, mainly in humic material.

Table 110: Concentration of carbendazim during aerobic incubation of treated test soils.

Days after application	mg carbendazim/kg dry soil	Carbendazim (% of concentration at day 0)
Standard soil 1 (sand)		
0	3.00	100
20	2.15	71.7
40	1.53	51.0
55	1.09	36.3
85	0.45	15.0
240	0.19	6.3
380	0.105	3.5
Standard soil 2 (loamy sand)		
0	3.00	100
10	2.57	85.7
25	1.87	60.3
75	0.83	27.7
120	0.57	19.0
240	0.28	9.3
380	0.16	5.3
480	0.11	3.7

The DT_{50} values was determined graphically to be approximately 40 days in both soils. Optimized degradation rates were derived by means of Model MakerTM vers. 4.0 fitting runs and were tested with regard to the acceptability of SFO kinetics in accordance with FOCUS kinetics guidance (2006) (Zillgens, 2007a), see table below.

Table 111: Re-calculated laboratory DT50 and DT90 for the aerobic degradation of carbendazim in two soils.

Soil	Number of data points	Temp (°C)	Moisture	DT_{50} (days)	DT_{90} (days)	$\chi 2$, %-error	Model
Sand	7	22	40% MWHC	37	123	7	SFO
Loamy sand	8	22	40% MWHC	44	146	9	SFO

RMS conclusion

The soil properties and study conditions are poorly described, but it can be consistently shown that 2-AB or other extractable residues account each for less than 5% of the applied radioactivity throughout the duration of the experiment. The mineralization rate amounts to maximal 12 - 19% (based only on recovery of carbendazim, not specific measurements of $^{14}CO_2$). Non-extractable residues are not detected quantitatively, but it is suspected that their proportion is relatively high.

Study summary derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany:

Reference:	**1. Gildemeister, H. Jordan, H.J. and Remmert, U (1981)** Behaviour of the plant protection product HOE 17411 OF AT 102 (Carbendazim) in soil SS 2.2 at 15 °C, 20 °C and 25 °C **2. Zillgens, B. (2007a)** Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model MakerTM 4.0
Report No.:	1 (B) 125/81; A16743, BOD95- 00446 2. DuPont-21656
Guideline:	BBA leaflet no. 36
GLP:	1. No
Validity	1. Valid

Material and methods:

An aerobic soil degradation study was conducted according to BBA leaflet no. 36. (2-^{14}C) 2-(methoxycarbonylamino)-benzimidazole in the formulation Hoe 17411 OF AS 101 was added to a sandy soil at a concentration of 2.674 mg carbendazim/kg corresponding to 711.3 g/ha. The amount of applied active substance is approximately 1.5-fold higher than the maximum application rate of 2 x 250 g as/ha. The specific activity was 16.45 MBq/g.
The study was conducted with one sandy soil at 3 different incubation temperatures. A summary of the physical and chemical properties of the soil is provided in the below table.
100 g soil was treated with 2.674 mg/kg Carbendazim in separate containers. All soil samples were adjusted to 40 % maximum water holding capacity (MWHC) and were incubated in darkness at 15, 20 and 25°C for 28 days.
Samples were taken at time intervals 0, 7, 14, 21 and 28 days for subsequent analysis.
The samples were extracted with methanol/glacial acetic acid/hydrochloric acid (0.1M) (90+10+5 (v/v/v)). Subsequently the neutralisation with saturated $NaHCO_3$ solution and a re-extraction with ethyl acetate was carried out. For the quantification of Carbendazim in the residues of soil the ^{14}C-radioactivity was detected by liquid scintillation counting following TLC separation with silica gel (solvent: methylene chloride/methanol 9+1 (v+v). The lower limit of determination was 0.01 mg/kg.

Kinetic analyses: DT_{50}/DT_{90} were determined by linear regression in the original report (Gildemeister et al, 1981). DT_{50}/DT_{90} were re-calculated in accordance with FOCUS guidance (in Zillgens, 2007a) and those results were the final ones.

Table 112: Soil properties.

Soil property	SS 2.2 (Schwanheimer Sand)
Particle size distribution (DIN) (%)	
< 2 µm	37.0
Classification (BBA)	sand
Organic carbon (%)	2.7
pH ($CaCl_2$)	4.7
Maximum water holding capacity (w/w %)	n.a.

Results

The decrease of carbendazim during incubation period was summarized in the table below. Recovery (total radioactivity in extracts and bound residues) over the whole period of the study was between 76 and 90%. Because mineralisation was not detected, a mass balance was not completed for this study. Already on day 0 about 9.5% of the initial applied

radioactivity was no longer extractable. By the end of the study (day 28) this value had risen to 48- 56% depending on the test temperature. Radio-TLC investigation of the extracts at the different sampling points showed that over 92% of these consisted invariably of carbendazim. There were also marginal amounts of unidentified metabolites (three at most). These results indicate either that the active ingredient itself was adsorbed fairly rapidly or that other degradation products, if any, were present almost quantitatively as bound residues.

Table 113: Decrease of carbendazim during incubation in one soil at three different temperatues over a period of 28 days.

Days after application	Carbendazim (mg/kg)			Carbendazim (% of initial)		
	15ºC	20ºC	25ºC	15ºC	20ºC	25ºC
0	2.422	2.422	2.422	100	100	100
7	1.882	1.776	1.728	77.7	73.3	71.4
14	1.736	1.606	1.55	71.7	66.3	64.0
21	1.497	1.464	1.343	61.8	60.4	55.4
28	1.38	1.291	1.16	57.0	53.3	47.9

The DT_{50} values in the original study were determined by linear regression to be 28, 25 and 21 days at 15, 20 and 25°C with r^2 range of 0.96 – 0.98. Recalculated values (Zillgens, 2007a) are given in the table below. Optimized degradation rates were derived by means of Model MakerTM vers. 4.0 fitting runs and were tested with regard to the acceptability of SFO kinetics in accordance with FOCUS kinetics guidance (2006). A statistical analysis showed that the degradation of the parent could be generally well described by a SFO kinetic model.

Table 114: Re-calculated laboratory DT_{50} and DT_{90} for the aerobic degradation of carbendazim in sand soil at three different temperatures.

Soil	Number of data points	Temp (ºC)	Moisture	DT_{50} (days)	DT_{90} (days)	$\chi 2$, %-error	Model
Sand	5	15	40% MWHC	34	112	4	SFO
	5	20	40% MWHC	31	102	5	SFO
	5	25	40% MWHC	26	86	5	SFO

RMS conclusion

The soil properties and study conditions are poorly described, but it can be consistently shown that there were only marginal amounts of unidentified metabolites (three at most). So that it can be ascertained, that 2-AB or other extractable residues account each for less than 5% of the applied radioactivity throughout the duration of the experiment. The mineralization rate amounts to maximal 10 - 24% (based only on recovery of applied radioactivity in extracts and bound residues, not specific measurements of $^{14}CO_2$). Non-extractable residues account 48 - 56% depending on the test temperature.

The study results are plausible.

11.1.4.4 Photochemical degradation

The available data (summarised in RAR Volume 3, B.8.2.1.2) showed that thiophanate-methyl is photolytically degradable under outdoor conditions (Japan, mid-December), with a half-life of 2.3 days under the conditions of the test. The quantum yield (ϕ) was determined to be 3.84×10^{-3}. From this results DT_{50}s for water bodies and clear sky conditions at 40°N latitude were calculated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter). The RMS concluded that direct photochemical transformation may contribute to the degradation of thiophanate-methyl under environmental conditions, and that the major product formed is carbendazim, with DX-105 formed in less amounts. Photochemical transformation is not expected to be a significant route of degradation of carbendazim.

Reference:	1. **Shiotani, H. (2003c)** Photodegradation of Thiophanate-methyl. (A previous study by **Soeda & Shiotani (1987b**, Report No. EC-74, RD-8701 (712-001)) was evaluated and considered acceptable in the DAR (1997). The report from the same experiment was re-written by Shiotani (2003c) to fulfil US EPA requirements.) 2. **Kiesel, A. and Geibel, E. (2015g)** Assessment of degradation kinetics of Thiophanate-methyl in a study on aqueous photochemical degradation according to the recommendations of the FOCUS Report on Degradation Kinetics (2006, 2014). 3. **Kiesel, A. and Geibel, E. (2015h)** Raw data to the assessment of degradation kinetics for Thiophanate-methyl based on data of an aqueous photolysis study according to FOCUS Degradation Kinetics (2006, 2014) related to the Supplementary Dossier - M-CA Section 7.
Report No.:	1. RD-03185 2. RD-03367 3. RD-03368
Document No.:	1. 712-002 2. 782-039 3. 782-085
Guideline:	1. US EPA FIFRA guideline 161-2, and US EPA CG-6000 2. and 3. FOCUS degradation kinetics (2006, 2014)
GLP:	1. No 2.and 3. Not applicable
Previous evaluation:	All three studies submitted for the purpose of renewal.
Material and methods:	
Test material:	[Benzene-U-^{14}C] thiophanate-methyl
Lot/Batch No:	CFQ 4519
Radiochemical purity:	> 99% (TLC)
Test concentration:	10 mg/l (thiophanate-methyl was dissolved in chloroform and the solvent was evaporated before the material was dissolved in buffer)
Test system:	Quartz tubes >16 mL, sterile. Buffer (pH 5): acetate-acetic acid, passed through a 0.2 µm filter.
Test conditions:	Outdoors in Japan in mid-December (latitude 35°, longitude 139°E), (temperature and light conditions were provided in tables), 10 dark controls.
Sampling time points:	Single samples after 0, 0.5, 1, 2, 4, and 7 days. The dark control (two) samples analysed at 0.5 and 1, 2, 4 and 7 days.
Method of analysis:	Radioactivity in aliquots of the samples were determined by LSC. Characterisation was done by HPLC and TLC. Test solution was extracted twice with 10 dichloromethane. The extract was dried and dissolved in dichloromethane and spotted on thin layer plate and developed two-dimensionally. Major photoproducts were extracted from TLC-plates and purified by HPLC. Mass spectra of these extracts and corresponding standards were determined.

	Analysis for concentration of thiophanate-methyl was done as area under curve with HPLC with UV-detector. LOD was 5 ng.
Used standards:	Thiophanate-methyl, carbendazim (MBC), DX-105 and FH-432
Actinometer:	A standard p-nitroacetophenone-pyridine (PNAP/PYR) actinometer was used. Concentration of PYR was adjusted so that experimental half-lives of PNAP and thiophanate-methyl were similar. The solution was irradiated in parallel with the test solutions. After 0, 0.5, 1, 2, 4 and 7 days the concentration of PNAP in duplicate samples was determined by HPLC. Concentrations of thiophanate-methyl and PNAP were used to determine the quantum yield and estimate the rate constant for summer, spring and winter seasons.
Kinetic evaluation:	In addition to estimation of half-lives from the quantum yield the study report also estimated the half-life from the measured concentrations using first order kinetic. Degradation rates were re-assessed by Kiesel and Geibel (2015g,h), in accordance with FOCUS (2006, 2014). KinGUI version 2.1 was used with SFO and FOMC kinetic models. The best-fit kinetic model was selected on the basis of Chi2 error-%, t-test/confidence interval and visual assessment.

Results

Ambient temperatures varied between 2.9°C and 16.3°C during exposure (as determined 3 times per day).

The recovery ranged from 98.8% to 101.9% of applied radioactivity in both irradiated and dark control samples during the course of the study indicating that no volatile compounds evolved. No significant loss of thiophanate-methyl or PNAP was observed in controls, so the loss in irradiated samples were attributed solely to photolysis. The major degradation products carbendazim (MBC) and DX-105 reached 49.7 and 14.3% AR, respectively.

Table 115: Irradiated samples: Distribution and characterisation of radioactivity in aqueous samples with 14C-thiophanate-methyl irradiated outdoors in Japan. As % of applied radioactivity.

Fraction - compound	0 day	0.5 day	1.0 day	2.0 days	4.0 days	5.5 days
Extractable ^{14}C	98.8	98.9	100.0	97.0	93.9	91.8
TM	92.5	85.9	81.4	63.4	28.1	12.5
DX-105	2.0	3.2	6.3	8.5	11.5	14.3
FH-432	0	0	0	0	2.8	4.4
MBC	1.8	4.3	5.4	13.1	38.0	49.7
Unknown 1	0.6	0.7	1.0	2.0	3.5	2.5
Unknown 2	0	0	2.6	5.5	5.6	4.3
Others *	1.9	4.8	3.3	4.5	4.4	4.1
Aqueous ^{14}C	1.2	1.5	1.5	3.0	4.9	7.3
Total ^{14}C	100	100.4	101.5	100	98.8	99.1

* "Others" consisted of at least six minor compounds including those remaining at the TLC origin.

Table 116: Dark controls: Distribution and characterisation of radioactivity in aqueous samples with 14C-thiophanate-methyl kept outdoors in Japan. As % of applied radioactivity.

Fraction - compound	0 day	0.5 day	1.0 day	2.0 days	4.0 days	5.5 days
Extractable ^{14}C	-	99.5	100.8	99.1	98.8	98.6
TM	-	-	-	91.9	-	90.8
DX-105	-	-	-	2.0	-	2.0
FH-432	-	-	-	0	-	0
MBC	-	-	-	1.8	-	1.8
Others *	-	-	-	3.3	-	4.0
Aqueous ^{14}C	-	1.3	1.1	1.8	1.6	1.7
Total ^{14}C	-	100.8	101.9	100.9	100.4	100.3

* Others consisted of at least six minor compounds including those remaining at the TLC origin.

- No data available.

The quantum yield (ϕ) was determined to be 3.84 x 10^{-3}. From this results DT_{50}s for water bodies and clear sky conditions at 40°N latitude were calculated to 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter).
From the regression analysis of the measured concentrations DT_{50} was estimated to 2.2 days under the conditions of the test.

The results of the kinetic re-analyses of the data by Kiesel and Geibel (2015g,h) are summarised in the table below. After optimisation of parameters for the parent only, a pathway fit with flow from the parent to carbendazim and to DX-105 was done. Time zero amounts of transformation products were added to the parent compartment. SFO kinetics represented the best fit for thiophanate-methyl (Chi2 error-% was marginally higher in FOMC).Visual assessments were considered acceptable. Kiesel and Geibel (2015g,h) accepted the results for carbendazim and DX-105 and therefore selected the results from the pathway fit also for the parent. For thiophanate-methyl the RMS has instead selected the results from the parent only fit since the results for the transformation products were not acceptable statistically. For the same reason, no formation fractions are shown.

Table 117: Summary of kinetic re-analysis by Kiesel and Geibel (2015g,h) of the aquatic photolysis data from Shiotani (2003c). Irradiated buffered solution at pH 5 and ambient temperatures in December outdoors in Japan.

Substance	Kinetic model	Mo	Parameter	χ2, %-error	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Parent only									
Thiophanate-methyl	**SFO**	102.0	k= 0.2979	6.8	6.4 x 10^{-4}	0.2	0.4	2.3	7.7
Pathway fit									
Carbendazim	SFO-SFO	n.r	k=2.3 x 10^{-14}	24.8	0.5	-0.2	0.2	>1000	>1000
DX-105	SFO-SFO	n.r	k=0.06637	7.1	0.09	-0.03	0.2	10.4	34.7

n.r. Not relevant.

RMS comments and conclusion

The experimental study (Soeda & Shiotani, 1987b) was considered acceptable in the DAR (1997). The report was re-written by Shiotani (2003c) to fulfil US EPA requirements but the description of the experiment and the results were the same. The study is still considered acceptable. The experiment was conducted in compliance with a relevant guideline.

This was a non-GLP study but the experiments appeared scientifically sound. It is not considered likely that repetition of the study would alter the conclusion substantially. It was unclear whether duplicate samples were taken for analyses, but in any case results from individual replicates were not presented.

During peer review the variation in temperature came into question, since modern guidelines (OECD TG No 316) recommend a more stable temperature. However the RMS believes that within an environmentally realistic temperature range such variation is not expected to have any dramatic influence on the rate of photochemical reactions. In addition to estimation of half-life from the measured amounts of thiophanate-methyl, half-lives were also estimated from the quantum yield, and the results were fairly similar. So regarding rate of photolysis the RMS suggest that it can be concluded from the study that the photochemical DT_{50} may be in the order of 1-5 days (40°N) depending on season. (Evaluation Table, open point 4.11)

Concerning the relevance of the unknown product 2 the RMS notes that degradation rates were also relatively fast in microbially active systems ($DegT_{50}$s 0.6-3.5 days), and so was the dissipation rate from water ($DisT_{50}$s 1.6-2.8 days). The unknown photo-product 2 was measured as 5.5 and 5.6% AR at sampling points 2 and 4 days, and at the next (and last) samling point the amount was 4.3% AR. After 2-4 days in a biologically active natural system less than half of the initial amount of thiophanate-methyl would still be present. Therefore it is not expected that the unknowm photo-product 2 would be formed as >5% under natural conditions. Since the other major photo-products were identified and given the relatively fast biodegradation the RMS conclude that the available data on photochemical transformation is sufficient. (Evaluation Table, open point 4.11)

11.1.5 Summary and discussion on environmental degradation

In a ready biodegradability test in accordance with OECD TG 301C, biodegradation reached only 4% in 28 days. In degradation studies in water only, in water/sediment systems and in soil, the parent compound is transformed with half-lives from less than one day up to a few days to the major environmental metabolite carbendazim. Carbendazim on the other hand, is more persistent with half-lives of 64.8 days, of 15.1-91.6 days and of 22.0-63.2 days in water only, in water/sediment and in soil, respectively. Carbendazim was found as >80% in water and water/sediment studies and as >10% after only one day. In soil studies, carbendazim was found as max 75.8%. Additional metabolites were formed but to a lesser extent compared to carbendazim. Based on the available information, thiophanate-methyl is considered as not rapidly biodegradable.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant

11.3 Environmental fate and other relevant information

Not relevant.

11.4 Bioaccumulation

Table 118: Summary of relevant information on bioaccumulation.

Method	Results	Remarks	Reference
	No data, not needed.		

11.4.1 Estimated bioaccumulation

When experimental data on the bioaccumulation are absent, Kow can be used for non-ionised substances to estimate the potential for bioaccumulation according to recommendations in the Guidance on the Application of the CLP Criteria (ECHA-09-G-02-EN. 2009). Since the Log Kow values for thiophanate-methyl and the major metabolite carbendazin are ≤ 4, the EG Reg 1272/2008 cut-off value, the potential for bioaccumulation is considered to be low (actual value 1.45 and 1.5 for thiophanate-methyl and carbendazim, respectively, see section 7).

11.4.2 Measured bioaccumulation

No measured bioaccumulation data were available, and not needed.

11.5 Acute aquatic hazard

The available and reliable data on acute toxicity of thiophanate-methyl, its major environmental metabolite carbendazim and additional metabolites to aquatic organisms is summarised in the table below.

Table 119: Summary of relevant information on acute aquatic toxicity. Key data are marked in bold text

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Fish					
Oncorhynchus mykiss	Thiophanate-methyl	Acute 96 hr (flow-through)	Mortality, LC_{50}	11.0 (7.6 – 13) (mm)	Report number 821-001
Ictalurus punctatus	Carbendazim*	96 hr	Mortality, LC_{50}	**0.019** (nom)	Report number A30119; cited in EFSA (2010) *
Oncorhynchus mykiss	Carbendazim*	96 hr	Mortality, LC_{50}	0.19 (nom)	Report number A52914; cited in EFSA (2010) *
Oncorhynchus mykiss	4-OH-TM	96 h, static	Mortality, LC_{50}	>10 (mm, filtrated)	Report number 821-008
Oncorhynchus mykiss	CM-0237	96 h, static	Mortality, LC_{50}	>0.14 (mm, filtrated)	Report number 821-009
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	48 h, flow-through	EC_{50} NOEC	**5.4 (4.4-6.3)** (mm) <4.2 (mm)	Putt, A.E. (1992)
Daphnia magna	BAS 325 10 F	48 h, static	EC_{50} NOEC	4.4 (mm) 3.1 (nom)	Elendt-Schneider (1991)
Daphnia magna	Carbendazim*	48 h	EC_{50}	0.15 (nom)	Fisher (1988), cited in EFSA (2010) *
Daphnia magna	4-OH-TM	48 h, static	EC_{50}	>17.6 (mm, filtrated)	Fujikake, N. (2012)
Daphnia magna	CM-0237	48 h, static	EC_{50}	>0.27 (mm, filtrated)	Fujikake, N. (2012)
Chironomus riparius	4-OH-TM	48 h, static	EC_{50}	>14.6 (mm, filtrated)	Kley, A.. Wydra. V. (2012)
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	E_rC_{50}	37.2 (25.6 – 169) (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	E_rC_{50}	27.3 (22.4 – 33.2) (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	E_rC_{50}	> 11 mg/L (mm)	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	E_rC_{50}	> 15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	E_rC_{50}	> 0.182 (mm)	Baba, K. (2012)
Higher plant					
No data, not needed					

* Refer to the EFSA conclusion on the peer review of the active substance Carbendazim, EFSA (2010). The study summaries provided below for Carbendazim are derived from the Draft Re-Assessment Report produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not evaluated by Sweden at this stage.

11.5.1 Acute (short-term) toxicity to fish

Reference:	**xxxx (1993):** Thiophanate-methyl - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Report number:	RD-9330, 93-4-4738; 12681.1292.6101.108 (821-001)
Guideline:	US EPA 72-1
GLP:	Yes
Previous evaluation:	In DAR 1997.
Material and methods:	
Test material:	Thiophanate-methyl

Lot/Batch No:	Lot# TIF-01016 (non-radiolabelled), Lot# 109F9209 (radiolabelled)
Purity:	95.95±0.36% (non-radiolabelled), >98% radiopurity (radiolabelled)
Species:	Rainbow trout (*Oncorhynchus mykiss*), length 37 (30-48) mm, body weight 0.47 (0.28-0.75) g w.w.
Test system/test conditions:	Flow-through conditions. Twenty organisms (ten per replicate) were exposed in duplicate test aquaria to each of five concentrations of Thiophanate-methyl, a solvent control (dimethylformamide, DMF), and a dilution water control for 96-hours. The test solutions were prepared with a combination of non-radiolabelled and radiolabelled (^{14}C) test material. Nominal concentrations of 2.6, 4.3, 7.2, 12 and 20 mg a.i./L were maintained by introducing approximately 6.4 aquarium volumes per day of newly prepared test solution via an intermittent-flow proportional diluter apparatus. Each replicate solution was sampled and analyzed for Thiophanate-methyl concentration at 0-hour (test initiation) and 96-hours (test termination) of exposure. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Test temperature was 12±1°C, pH 6.8-7.2, oxygen saturation 80-90%. Hardness and alkalinity ranged 25-26 mg/L and 20-23 mg/L, respectively (as $CaCO_3$). Photoperiod was 16 hours light per day.

Results:

Based on the results of the chemical analyses, the mean measured exposure concentrations were defined as 2.4, 4.7, 7.6, 13 and 20 mg a.i./L, corresponding to 92, 109, 106, 108 and 100% of the nominal values.

Table 120: Concentration of 14C-Thiophanate-methyl measured in replicate test solutions during the 96 hour flow-through exposure of rainbow trout (*Oncorhynchus mykiss*)

Nominal Concentration mg a.i./L	**Measured Concentration after [mg a.i./ L]**				
	0-Hour		**96-Hour**		**Mean**
	A	**B**	**A**	**B**	
Control	<0.36	<0.36	<0.36	<0.36	
Solvent control	<0.36	<0.36	<0.36	<0.36	
2.6	2.4	2.4	2.3	2.4	2.4
4.3	4.6	4.7	5.1	4.3	4.7
7.2	7.1	7.9	7.5	7.9	7.6
12	13	13	13	13	13
20	20	21	20	20	20

After 96 hours, 100 and 75% mortality was observed at 20 and 13 mg a.i./L, respectively. At lower test concentrations, no mortality was observed. Sublethal effects were observed at 4.7 mg a.i./L and higher. The resulting 96 hour LC_{50} was determined to be 11 (7.6 – 13) mg a.i./L, and a NOEC could be set to 2.4 mg a.i./L based on mean measured test concentrations.

Table 121: Mortality during the 96 hour flow-through exposure of *Oncorhynchus mykiss* to Thiophanate-methyl

Mean measured Conc. [mg/L]	**Cumulative mortality(%)**											
	24-hour			**48-hour**			**72-hour**			**96-hour**		
	A	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
2.4	0	0	0	0	0	0	0	0	0	0	0	0
4.7	0	0	0	0	0	0	0	0	0	0	0	0
7.6	0	0	0	0	0	0	0	0	0	0	0	0
13	10	0	5	20	0	10	40	40	40	70	80	75
20	0	10	5	40	50	45	90	100	95	100	100	100

RMS comments:

The study was re-evaluated against the validity criteria of the corresponding OECD TG 203 (1992):

1. mortality in the control(s) should not exceed 10% at the end of the test;
2. constant conditions maintained as far as possible throughout the test (eg. semi-static or flow-through)
3. dissolved oxygen concentration at least 60 per cent of the air saturation value throughout the test;
4. concentrations of test substance were satisfactorily maintained, and preferably at least 80% of nominal.

All these criteria were fulfilled, and the study is considered to be valid and useful for the risk assessment.

Author: xxxx

Title: Toxicological Studies of Benomyl and Carbendazim in Fish

Date: Dec 1984

Doc ID: A30119; BVL No. 1758267

Guidelines: ASTM

GLP: Not applicable

Validity: Results considered to be valid

Methods

Conducted according to prevailing standards and subsequently accepted for international registration.

10 animals per tested concentration (0.3 g to 1.2 g)

Rainbow trout (*Oncorhynchus mykiss*): 12° C

Channel catfish (*Ictalurus punctatus*): 22° C

Bluegill (*Lepomis macrochirus*): 22° C

Test concentrations and application of test substance: Not reported; static testing

Additional series of acute tests were performed to determine the effects of temperature (rainbow trout 7° C, 12° C and 17° C; channel catfish: 12° C, 17° C and 22° C), pH (rainbow trout and channel catfish: 6.5, 7.5 and 8.5), hardness (rainbow trout: soft water of pH 8, hardness 40 mg/l $CaCO_3$ and hard water of pH 8, hardness 320 mg/L $CaCO_3$) and exposure of early life stages (rainbow trout and channel catfish) yolk-sac fry, swim-up fry and 0.2 g fry.

Results

Table 122: Acute toxicity of carbendazim for rainbow trout (Oncorhynchus mykiss) channel catfish (Ictalurus punctatus) and bluegill (Lepomis macrochirus):

Species	Endpoint	Value (mg/L)	time	ai	n/r/i
Oncorhynchus mykiss	LC_{50}	0.87 (0.63-1.19)	96 h		n
Lepomis macrochirus	LC_{50}	>3.2	96 h		n
Ictalurus punctatus	LC_{50}	0.019 (0.013-0.027)	96 h		n

n = nominal; r = real; i = initial

Table 123: Acute toxicity of carbendazim for *Ictalurus punctatus* –results of additional test series

Early life stages	LC_{50} (mg/L)
yolk-sac fry:	0.007 (0.006 - 0.009)
swim-up fry :	0.012 (0.009 - 0.015)
0.2 g fry:	0.01 (0.008 - 0.013)
water hardness	
40 mg $CaCO_3$	0.018 (0.011 - 0.028)
320 mg $CaCO_3$	0.024 (0.018 - 0.032)
pH	
6.5	0.023 (0.018 - 0.031)
7.5	0.014 (0.011 - 0.018)
8.5	0.023 (0.018 - 0.029)
temperature	
12° C	> 0.56
17° C	0.14 (0.091 - 0.216)
22° C	0.032 (0.023 - 0.044)

Table 124: Acute toxicity of carbendazim for Oncorhynchus mykiss –results of additional test series

Early life stages	**LC_{50} (mg/L)**
yolk-sac fry:	0.145 (0.109 - 0.192)
swim-up fry :	0.32 (0.24 - 0.43)
0.2 g fry:	0.37 (0.27 - 0.51)
water hardness	
40 mg $CaCO_3$	0.78 (0.58 - 1.05)
320 mg $CaCO_3$	0.88 (0.64 - 1.21)
pH	
6.5	0.64 (0.46 - 0.90)
7.5	0.41 (0.31 - 0.44)
8.5	0.34 (0.26 - 0.44)
temperature	
7° C	> 1.8
12° C	0.87 (0.63 - 1.19)
17° C	0.10 (0.07 - 0.14)

Conclusion

Report results listed and evaluated in the carbendazim monograph of 13.11.1997. The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfil the objectives of the study and there is no reason to doubt the validity of the results. Critical point: raw data missing (esp. control treatments). Determined median lethal concentrations after 96 h are for *Oncorhynchus mykiss* LC_{50} = 0.87 mg carbendazim/L, for *Lepomis macrochirus* LC_{50} > 3.2 mg carbendazim/L and for *Ictalurus punctatus* LC_{50} = 0.019 mg carbendazim/L.

Author: xxxx

Title: Acute (96-hour) toxicity of H-9910 to rainbow trout

Date: Jun 1976

Doc ID: A52914, Report no: HLO486-76; BVL No. 1840602

Guidelines: Not applicable

GLP: Not applicable

Validity: Results considered to be valid

Methods

Conducted according to prevailing standards and subsequently accepted for international registration. The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfil the objectives of the study and there is no reason to doubt the validity of the results.

Test species: Rainbow trout (Oncorhynchus mykiss). Number of animals: 10 per tested concentration (static, stock solution in acetone)

control, 0.10, 0.14, 0.24, 0.32, 0.42, 0.56, 0.75, 1 and 2.1 mg/L

Maintenance: 15 L tanks; loading: 3.7 cm fish/L; 12° C ±1.0° C. Duration of exposure: 96 h

Observed parameters: Mortality and symptoms every 24 hours after start of exposure

Results

The reported values are based on nominal concentrations.

Table 125: Observed mortality in *Oncorhynchus mykiss* during exposure to carbendazim

Concentrations	Survival (No. animals)		
Control	10	10	10
0.10	10	10	10
0.14	10	10	9
0.24	10	10	2
0.32	10	9	0
0.42	10	9	0
0.56	10	10	0
0.75	10	3	0
1.4	10	1	0
2.1	10	0	0

Table 126: Acute toxicity of carbendazim for Oncorhynchus mykiss as given in the report summary and recalculated LC_{50}-values

Species	Endpoint	Value (mg/L) in the report	Value (mg/L) recalculated	time	ai	n/r/i
Oncorhynchus mykiss	LC_{50}	> 2.1	> 2.1	24 h		n
	LC50	0.84 (0.55 – 1.30)	0.75 (0.61-0.93)	48 h		n
	LC_{50}	0.34 (0.26 – 0.45)	0.19 (-)	96 h		n

n = nominal; r = real; i = initial

Conclusion

Report results not listed in the carbendazim monograph of 13.11.1997. The LC_{50} (96 h) of carbendazim, technical, in an acute static test with rainbow trout was reported to be 0.34 mg/L. Recalculated values according to the data given in the summary of the study report are LC_{50} (48 h) = 0.75 mg carbendazim/L and LC_{50} (96 h) = 0.19 mg carbendazim/L.

Reference:	**Kley, A., Wydra, V. (2012):** Acute Toxicity of 4-OH-TM to Rainbow trout (*Oncorhynchus mykiss*) in a 96-hour Static Test
Report number:	RD-02401, 67761230 (821-008)
Guideline:	OECD TG 203 (1992)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	4-OH-TM (Thiophanate-methyl metabolite)
Lot/Batch No:	31-10193-N.UMEDA
Purity:	95.3 %
Species:	Rainbow trout (*Oncorhynchus mykiss*), juveniles; average length 4.84 ± 0.2 cm; average weight 1.09 – 0.16 g
Treatments:	Nominal concentrations: 0, 4.3, 9.4, 21, 45 and 100 mg/L
Controls:	Reconstituted water
Replication:	Seven fish each were prepared for each experimental group.
Duration:	96 hours
Test conditions:	Static test conditions. Temperature 13 – 14°C; pH:7.7 – 8.0 Hardness: 250 mg/L as CaCO3; Conductivity ≤ 10 µS/cm Photoperiod 16 h light : 8 h dark; Light intensity 490 – 950 lux Fish were fed three times per week or daily until 24 h prior to test initiation or during the exposure period. The toxicity test was conducted in 12 L glass aquaria, containing 10 L of test solution.
Observations:	Records of mortality and sublethal effects of exposure were made after 0, 2, 24, 48, 72 and 96 hours.
Chemical analysis:	Test substance concentrations were analysed at the test start and end were analysed via HPLC-UV method. LOD was 0.025 mg/L and LOQ was 1 mg/L.
Data analysis:	The determined endpoints were the NOEC and LOEC after 96 h as well as the LC_{50} after 96 h.

Results:

Analytical results

At test start, turbidity of the test medium caused by the test item was observed at nominal 21, 45 and 100 mg/L, with the strongest turbidity observed at 100 mg/L. After 2 hours of test duration, some of the test item was lying at the bottom of the aquaria of all test item treatment groups and the test medium of the 21, 45 and 100 mg/L test item treatment groups was still turbid. After 24, 48, 72 and 96 hours of duration, the test item was still observed at the bottom of the aquaria of all test item treatment groups and the test medium of the 45 and 100 mg/L test item treatment groups was still turbid.

At the start of the test, on average 95 % of the nominal concentrations were found in the non-filtrated samples. After 96 hours, on average 51 % of the nominal concentrations were determined in the non-filtrated samples. These results were caused by settlement of the test item during the test (with exception of the lowest test item concentration).

In the filtrated samples, on average 23 % of the nominal concentrations were found at the start of the test and 46 % were found at test termination. Hence, the concentration of dissolved test item increased during the course of the test. This was however not the case at the highest treatment level, where all measured values were 10% of the nominal test concentration.

All reported biological results were related to the mean measured concentrations of the test item in non-filtrated samples.

Table 127: Arithmetic mean measured 4-OH-TM concentrations in the exposure solutions during the 96 h exposure of rainbow trout

Nominal concentration [mg/L]	Mean measured concentration [% of nominal] [1]			
	Non-filtrated samples	Standard deviation	Filtrated samples	Standard deviation
0	n.a.	n.a.	n.a.	n.a.
4.3	94	3	58	56
9.4	85	15	49	52
21	68	38	32	32
45	62	66	20	9
100	55	99	10	0

[1] *Arithmetric mean value of all measured samples per treatment group.*

n.a. = not applicable

Measurements of pH, dissolved oxygen and temperature were unaffected by the concentrations of 4-OH-TM tested and remained within acceptable ranges.

Biological results

In the control and all treatment groups, all fish survived until the end of the experiment. After 2 hours of exposure, one fish of the nominal 100 mg/L test item treatment group was observed to be swimming mainly at the bottom of the aquarium. No other sublethal effects or signs of intoxication were observed. Since no mortality was observed in any of the test item treatment groups, the 96 h LC_{50} was empirically estimated to be > 55 mg/L based on mean measured concentrations (non-filtrated samples). The NOEC was determined to be at least 55 mg/L.

RMS comments:

The study was well performed and reported. The study was evaluated against the validity criteria of OECD TG 203 (1992):

1. mortality in the control(s) should not exceed 10% at the end of the test;
2. constant conditions maintained as far as possible throughout the test (eg. semi-static or flow-through)
3. dissolved oxygen concentration at least 60 per cent of the air saturation value throughout the test;
4. concentrations of the test substance satisfactorily maintained, and preferably at least 80% of nominal.

Except for the fourth criterium regarding the test substance satisfactorily maintained, these criteria were fulfilled. However, this was compensated by using the analytically measured test concentration for the results. Hence, the study is considered to be valid and useful for the risk assessment.

It was noted that the measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 96 hour LC_{50} of >10 mg/L.

Reference:	**Kley, A., Wydra, V. (2012):** Acute Toxicity of CM-0237 to Rainbow trout (*Oncorhynchus mykiss*) in a 96-hour Static Test
Report number:	RD-02403, 67771230 (821-009)
Guideline:	OECD TG 203 (1992)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	CM-0237
Lot/Batch No:	31-11145-S.KOIZUMI
Purity:	99.1%
Species:	Rainbow trout (*Oncorhynchus mykiss*), Juveniles; average length 4.84 ± 0.2 cm; average weight 1.09 – 0.16 g.
Treatments:	Control, 4.3, 9.4, 21, 45 and 100 mg/L (nominal)
Replication:	7 individuals per treatment group
Controls:	Reconstituted water
Duration:	96 hours
Test conditions:	Static test. Temperature: 13 – 14°C, Dissolved oxygen: 95 – 101 % of air saturation.
	pH: 7.7 – 8.0; Hardness: 250 mg/L as CaCO3
	Conductivity: ≤ 10 µS/cm
	Photoperiod: 16 h light : 8 h dark
	Light intensity: 490 – 1000 lux
Observations:	Records of mortality and sublethal effects of exposure were made after 0, 2, 24, 48, 72 and 96 hours.

Chemical analysis:	Test substance concentrations were analysed at the test start and end were analysed via HPLC-UV method. LOQ 1 mg/L.
Data analysis:	Due to the low mortality, no LC_{50} could be calculated.

Results:

Analytical results

Turbidity of the test medium caused by the test item was observed in all test item treatment groups during the whole test. Additionally, the test item was swimming at the water surface and lying at the bottom of the aquaria of the 21, 45 and 100 mg/L test item treatment groups during the whole test. In the 4.3 and 9.4 mg/L test item treatment groups, some test item was lying at the bottom of the aquaria after 24 hours until the end of the test.

At the start of the test, 91 % of the nominal concentrations were found in the non-filtrated samples. After 96 hours, on average 26 % of the nominal concentrations were determined in the non-filtrated samples. In the filtrated samples, the concentrations of the test item were above the limit of detection (0.006 mg/L) but below the limit of quantification (1 mg/L), which indicates the low water solubility of the test item. Reported biological results below are related to the mean measured concentrations of the test item (non-filtrated samples).

Table 128: Mean measured CM-0237 concentrations in the exposure solutions during the 96 h exposure of rainbow trout

Nominal concentration [mg/L]	**Mean measured concentration [% of nominal]** [1]			
	Non-filtrated samples	**Standard deviation**	**Filtrated samples**	**Number of samples**
0	n.a.	n.a.	n.a.	2
4.3	59	60	< LOQ (0.067 mg/L)	4
9.4	59	68	< LOQ (0.087 mg/L)	4
21	61	53	< LOQ (0.092 mg/L)	4
45	55	65	< LOQ (0.10 mg/L)	4
100	58	77	< LOQ (0.14 mg/L)	4

[1] *Mean value of all measured samples per treatment group*

n.a. = not applicable

Measurements of pH, dissolved oxygen and temperature were unaffected by the concentrations of CM-0237 tested and remained within acceptable ranges for the survival of *O. mykiss*.

Biological results

In the control and all test item treatment groups, all fish survived until the end of the experiment. No signs of intoxication were observed.

RMS comments:

The study generally fulfilled the validity criteria of OECD TG 203 (1992). However, the results must be regarded as uncertain due to the unknown bioavailability of the test substance since the dissolved portion of the test substance was below the limit of quantification at all treatment levels. The RMS proposes that, although uncertain, the 96 hour LC_{50} can be estimated to the mean measured concentrations (higher than LOD, below LOQ, therefore uncertain) at the highest treatment level, or >0.14 mg/L. Given that no mortality was observed in the test, this approach is considered as sufficiently protective for the risk assessment.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Reference:	**Putt, A.E. (1992):** Thiophanate-methyl - Acute toxicity to Daphnids (Daphnia magna) under flow-through conditions (FIFRA Guideline no.: 72-2)
Report number:	92-4-4217; 12681.1191.6100.115. RD-9229 (822-004)
Guideline:	U.S. EPA 72-2
GLP:	Yes
Previous evaluation:	In DAR 1997.
Material and methods:	
Test material:	Non-radiolabelled and radiolabelled thiophanate-methyl.
Lot/Batch No:	TIF-01016 and 109F9209
Purity:	97.57% for non-radiolabelled. >98% for radiolabelled.
Species:	*Daphnia magna*, less than 24 hours old.
Treatments:	3.1, 5.2, 8.6, 14 and 24 mg/L (nominal).
Replication:	Two replicates per treatment and controls, ten daphnids per test level.
Controls:	Untreated and solvent (DMF) control
Duration:	48 hours
Test conditions:	Flow-through test. Temperature: 18-22°C Dissolved oxygen: >60% of saturation pH: 7.8 - 8.3 Photoperiod: 16 h light : 8 h dark Light intensity: 30-50 footcandles
Observations:	Number of immobilised daphnids were monitored after 24 and 48 hours.
Chemical analysis:	At start and end of exposure, by means of LSC.
Data analysis:	Moving angle and probit analysis.

Results:

Mean measured test concentrations were 4.2, 5.3, 9.5, 15 and 24 mg/L, or 100 – 135% of the nominal values. The results were based on mean measured values.

Table 129: Concentration of ^{14}C-Thiophanate-methyl measured in replicate test solutions during the 48 hour flow-through exposure of daphnids (*Daphnia magna*)

Nominal Concentration mg a.i./L	**Measured Concentration after [mg a.i./ L]**				
	0 hour		**48 hour**		**geomean**
	A	**B**	**A**	**B**	**(SD)**
Control	<0.29	<0.29	<0.29	<0.29	
Solvent control	<0.29	<0.29	<0.29	<0.29	
3.1	4.2	4.3	4.1	4.1	4.2
5.2	5.4	5.4	5.1	5.2	5.3
8.6	9.6	10	9.1	9.4	9.5
14	16	15	14	15	15
24	23	24	24	23	24

The cumulative percent immobilisation is summarised in the table below.

Table 130: Cumulative percent of immobilised *Daphnia magna* exposed to Thiophanate-methyl

Mean Measured Cone. of Thiophanate-methyl [mg/1]	**Cumulative percent immobilised organisms**					
	24 hours			**48 hours**		
	A	**B**	**Mean**	**A**	**B**	**Mean**
24	60	60	60	100	100	100
15	80	40	60	100	90	95
9.5	40	60	50	100	100	100
5.3	20	0	10	60	40	50
4.2	0	0	0	30	10	20
Solvent Control	0	0	0	0	0	0
Control	0	0	0	0	0	0

In addition, sublethal effects (e.g. lethargy) were observed among all of the mobile daphnids exposed to the 15, 5.3 and 4.2 mg/L test concentrations. The 24 and 48 hour EC_{50} values were 14 (95% CL 11-20) and 5.4 (95% CL 4.4-6.3) mg/L, respectively. NOEC was <4.2 mg/L, due to the observed sublethal effects (lethargy) at all treatment levels.

RMS comments:

The study was re-evaluated against the validity criteria of OECD 202:

– less than 10% immobilization in the control

– dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled and the study is considered as still valid for risk assessment.

Reference:	**Elendt-Schneider (1991):** Determination of the acute toxicity of BAS 325 10 F on the water flea Daphnia magna STRAUS
Report number:	1/90/1119/50/1; 1991/10277; RD-00035 (822-001)
Guideline:	OECD TG 202 (1984)
GLP:	GLP, unpublished
Previous evaluation:	In DAR 1997.
Material and methods:	
Test material:	BAS 325 10 F, 500 g/L Thiophanate-methyl
Lot/Batch:	90-1
Test species:	Water flea (*Daphnia magna* STRAUS), neonates at test initiation 2 to 24 hours old
Test design:	Static system (48 hours), 7 treatment groups (6 test item rates, control), 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Treatments:	Control, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg formulation/L (nominal).
Replication:	5 animals per vessel, 20 per test concentration.
Test conditions:	Dilution water "M4" Elendt medium; test volume 10 mL, temperature 20-21°C; pH 7.8 - 8.1; oxygen content: 8.4 mg/L - 9.5 mg/L; total hardness: 2.2-3.2 mmol/L at test initiation; photoperiod: 16 h light : 8 h dark, light intensity: 5 - 6 µE/(m²*s); no feeding, no ventilation.
Chemical analysis:	The test item concentrations were analyzed using reversed-phase HPLC with UV-detection.
Data analysis:	Descriptive statistics, moving average method for determination of the EC_{50} values.

Results:

Mean measured concentrations were in the range 42.3 – 97.5% of the nominal values. Mean measured concentrations and the biological results are given in the table below.

Table 131: Results from acute toxicity study with Daphnia magna

Nominal test concentration (mg/L)	Mean measured test concentration (mg/L)	Number of live daphnids 24 hours (%)	Number of live daphnids 48 hours (%)
Control	Not detectable	20	20
0.39	Not analysed	20	20

Nominal test concentration (mg/L)	Mean measured test concentration (mg/L)	Number of live daphnids 24 hours (%)	Number of live daphnids 48 hours (%)
0.78	0.48 (61.5%)	20	20
1.56	Not analysed	20	20
3.13	Not analysed	20	20
6.25	Not analysed	20	19
12.5	10.1 (80.8%)	18	6
25	Not analysed	16	3
50	Not analysed	16	3
100	95.3 (95.3%)	14	0

The 48 hour EC_{50} value was 11.0 (95% CL 9.3-12.1) mg/L, corresponding to 5.5 (4.7 – 6.1) mg a.s./L. The 48 hour NOEC was set to 3.13 mg/L (corresponding to 1.6 mg a.s./L) due to the observed effects at all higher treatment levels.

RMS comments:

The study was re-evaluated against the validity criteria of OECD 202 (2004):

– < 10% immobilization in the control

– dissolved oxygen concentration > 60% of saturation

These validity criteria were fulfilled and the study is considered as still valid for risk assessment.

Measured test concentrations were below 80% of the nominal values within the test period. Therefore the results should be based on mean measured values. Since only three treatment levels were analysed, it is however not possible to re-calculate the EC_{50} value. Instead, it is proposed that the mean measured at the level closest to the 50% effect level is used for estimation of a mean measured EC_{50}, ie. 80.8% at 12.5 mg formulation/L. Hence, the 48 hour EC_{50} is 8.9 mg formulation/L (80.8% of 11.0 mg /L), corresponding to ca 4.4 mg a.s./L.

Author:	**Fischer, R.**
Title:	The Effect of Carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Daphnia magna* (waterflea) in a static-acute toxicity
Date:	04 Aug 1988
Doc ID:	A39285; Report no: OEK88/091E; BVL No. 1758297
Guidelines:	OECD 202
GLP:	Yes
Validity:	Yes

Aim was to assess the effect of carbendazim - substance, technical (identification code: Hoe 017411 OF ZD99 0010) to *Daphnia magna* (water flea) in a static-acute toxicity test (method OECD).

Methods

Test material: carbendazim; substance, technical; Code: Hoe 017411 OF ZD99 0010

Specification: Analytical certificate no. AZ 03819, dated 25 March 1988; batch 3/86; purity 99.4 %, solid, white crystalline powder. Stability of the test substance was shown by chemical analysis of test medium. Dosing vehicle was 37 % HCl or acetone.

Guideline: OECD 202 (1984)

Animals: *Daphnia magna* was bred by the testing laboratory. Number of animals in the test: 2 x 10 animals per tested concentration (6 x 10 in the range of 0.001 to 10 mg/L) and control. Age: max. 24 hours; maintenance conditions: 300 ml glass jars filled with 200 ml medium; 20° C ±1° C; photoperiod 16 h light.

Test medium: 70 % filtered (sand and activated charcoal) and 30 % deionised water

Test concentrations:

25 concentrations between 0.0010 mg/L and 1000 mg/L

Stock solution (in HCLlor acetone) added to the test medium; at 180 to 1000 mg/L, the test substance was directly added to the medium.

Duration of exposure/recording: 48 hours

Measured/Observed parameters: Immobilization: 24 and 48 hours

Chemical analysis of test substance: 0 and 48 hours, analytical monitoring: HPLC

Results

Due to varying results when acetone was used as a solvent and a recovery of > 80 % of nominal concentration when HCL was taken, all concentrations refer to nominal values. Data on immobilization and the calculated EC_{50} values are given in the tables below.

In the test systems with Acetone as solvent, the NOEC ranged from 0.032 (test system C) to 0.0056 (test system B). However, in these experiments the substance recovery ranged between 16 and 3247 %.(vierstellige %-Zahl ?)

Results of test system A (HCl as solvent) have to be regarded as invalid, since the control vessel showed a contamination with the tested substance when analyzed, even if no immobilization of the daphnids was observed. Report, page 14 '… In spite of all analytical ambiguities, the biological responses were quite comparable'.

In the test systems C with HCLlas solvent, the NOEC for *Daphnia magna* exposed for 48 h to carbendazim was 0.010 mg/L.

Table 132: Immobilization of *Daphnia magna* when exposed to carbendazim in an acute test

Concentration (mg/L)	Immobilization (%)							
	Test system A (HCL)		Test system B (A)		Test system C (A)		Test system C (HCL)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
control	0	0	0	0	0	0	0	0
HCI control	0	0	-	-	-	-	0	0
Acetone control	-	-	0	0	0	0	-	-
0.0010	-	-	0	0	0	0	0	0
0.0018	-	-	0	0	0	0	0	0
0.0032	-	-	0	0	0	0	0	0
0.0056	-	-	0	0	0	0	0	0
0.010	-	-	0	5	0	0	0	0
0.018	-	-	0	5	0	0	0	5
0.032	-	-	0	10	0	0	5	5
0.056	-	-	0	0	5	5	0	0
0.10	0	35	0	0	0	0	0	0
0.18	0	90	15	40	65	100	20	80
0.32	10	95	45	70	70	100	35	100
0.56	35	100	65	100	70	100	50	100
1.0	30	100	95	100	100	100	35	100
1.8	25	100	95	100	95	100	85	100
3.2	55	100	100	100	80	100	90	100
5.6	90	100	100	100	95	100	85	100
10	85	100	100	100	100	100	90	100
18	100	100	-	-	-	-	-	-
32	100	100	-	-	-	-	-	-
56	100	100	-	-	-	-	-	-
100	100	100	-	-	-	-	-	-
180	100	100	-	-	-	-	-	-
320	100	100	-	-	-	-	-	-
560	100	100	-	-	-	-	-	-
1000	100	100	-	-	-	-	-	-

HCl = Hydrochloric acid min. 37 % as solvent, A = acetone as solvent

Table 133: EC50 values for Daphnia magna following exposure to carbendazim

Endpoint	Value (mg/L)	95 % confidence interval (mg/L)	time	solvent	ai	n/r/i
EC_{50}	0.15	0.10 - 0.18	2 d	HCl		n
EC_{50}	0.80	0.59 - 1.12	1 d	HCl		n
EC_{50}	0.13	0.10 - 0.18	2 d	Acetone		n
EC_{50}	0.16	0.10 - 1.0	1 d	Acetone		n

n = nominal; r = real; i = initial

Conclusion

Report results listed and evaluated in the carbendazim monograph of 13.11.1997. For Daphnia magna exposed to carbendazim in a static test system, the EC_{50} (48 h) was 0.15 mg/L.

Reference:	**Fujikake, N. (2012):** 4-OH-TM: Acute immobilization Study in Daphnia magna
Report number:	RD-02410. NCAS 11-179 (822-007)
Guideline:	OECD 202 (1984)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	4-OH-TM
Lot/Batch No:	31-5267-DT
Purity:	99.2%
Species:	*Daphnia magna*, less than 24 hours old.
Treatments:	Control, 25, 50 and 100 mg/L (nominal)
Controls:	Elendt M4 medium
Duration:	48 hours
Test conditions:	Static test. Temperature: 20 ± 1°C Dissolved oxygen: 7.5 – 7.9 mg/L (87 - 89%) pH: 7.8 – 8.0 Photoperiod: 16 h light : 8 h dark Light intensity: 520 – 880 lux
Observations:	The number of immobilised D. magna in each replicate test vessel was recorded at 24 and 48 h of exposure. Immobilisation was defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made

	and recorded at 0, 24 and 48 h.
Chemical analysis:	The dissolved test substance concentrations (dissolved fluid concentrations) and the dissolved and non-dissolved but suspended test substance concentrations (suspended fluid concentrations) were measured with HPLC at test initiation and end.
Data analysis:	Since the concentrations of 4-OH-TM tested did not produce ≥ 50 % immobilisation, the EC_{50} was empirically estimated to be greater than the mean measured concentration for the highest treatment level tested.

Results:

Analytical results

The test medium was stained slightly milky white or milky white in a concentration-dependent manner at the test initiation in all test item treatment groups. The test medium became clear and colourless in all test item treatment groups at the end of the exposure period. White precipitates were observed in all of the exposure groups after 24 and 48 hours of exposure.

Table 134: Measured concentrations of 4-OH-TM in the exposure solutions during the 48 h exposure of *Daphnia magna*

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	**48 h**	**Mean**	**% of nominal**
Suspended fluid				
0	< LOQ	< LOQ	n.a.	n.a.
25	20.9	12.1	16.1	64.4
50	38.6	15.8	25.5	51.1
100	50.4	17.5	31.1	31.1
Dissolved fluid				
0	< LOQ	< LOQ	n.a.	n.a.
25	13.6	11.7	12.6	50.5
50	16.3	14.6	15.4	30.9
100	18.7	16.6	17.6	17.6

LOQ = limit of quantification. i.e. 0.1 mg/L

n.a. = not applicable

The suspended fluid concentrations of the test substance were 50.4 – 83.6 % of the nominal concentrations at test initiation and 17.5 – 48.4 % at the end of the test. The mean concentrations during the exposure period were 16.1, 25.5 and 31.1 mg/L in the 25, 50 and 100 mg/L test item treatment groups, respectively. The dissolved fluid concentrations of the test substance were 13.6, 16.3 and 18.7 mg/L at test initiation and 11.7, 14.6 and 16.6 mg/L at the end of the test in the 25, 50 and 100 mg/L test item treatment groups, respectively.

All chemical and physical parameters (dissolved oxygen concentration, pH. and temperature) in the definitive test were within expected ranges.

Biological results

In the latest test with the reference substance, the EC_{50} value determined in three tests was 0.66 – 1.3 mg/L.

The rate of immobilisation was 45 % in the 100 mg/L test item treatment group after 48 hours of exposure. Thus, the EC_{50} was estimated to be > 31.1 mg/L based on suspended fluid (mean measured concentration) and > 17.6 mg/L based on dissolved fluid (mean measured concentration).

Table 135: Immobilisation of *Daphnia magna* exposed to 4-OH-TM for 48 h in a static test

Nominal concentration [mg/L]	**Cumulative number of immobilised daphnids**	
	After 24 hours	After 48 hours
0	0	0
25	0	0
50	0	4
100	0	9

Since the concentrations tested did not result in ≥ 50 % immobilization, the 48 h EC_{50} value for *D. magna* exposed to 4-OH-TM was empirically estimated to be greater than the highest test concentration (> 31.1 mg/L. mean measured concentration when based on suspended fluid and > 17.6 mg/L, mean measured concentration when based on dissolved fluid).

RMS comments:

The study was well performed and reported. The study was evaluated against the validity criteria of OECD 202:

– less than 10% immobilization in the control

– dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled.

The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the geomean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC_{50} of >17.6 mg/L.

Reference:	**Fujikake. N. (2012):** CM-0237: Acute immobilization Study in Daphnia magna
Report number:	RD-02409. NCAS 11-178 (822-006)
Guideline:	OECD TG 202 (1984)

GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	CM-0237 (Thiophanate-methyl metabolite)
Lot/Batch No:	31-02122-I.TAKEUCHI
Purity:	98.1 %
Species:	Water flea (*Daphnia magna*), < 24 hours old
Treatments:	0 (control), 25, 50 and 100 mg/L (nominal)
Controls:	Elendt M4 medium
Replication:	Four replicate test vessels with five individuals each
Duration:	48 hours
Test conditions:	Static test design Temperature: 20 ± 1°C Dissolved oxygen: 7.3 – 7.9 mg/L (83-90% of saturation) pH: 7.8 – 8.0 Photoperiod: 16 h light : 8 h dark Light intensity 500 – 900 lux
Observations:	The number of immobilised D. magna in each replicate test vessel was recorded at 24 and 48 h of exposure. Immobilisation was defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 h.
Chemical analysis:	The dissolved test substance concentrations (dissolved fluid concentrations) and the dissolved and non-dissolved but suspended test substance concentrations (suspended fluid concentrations) were measured with HPLC at test initiation and end.
Data analysis:	Since the concentrations of CM-0237 tested did not produce ≥ 50 % immobilisation, the EC_{50} was empirically estimated to be greater than the mean measured concentration for the highest treatment level tested.

Results:

Analytical results

The test medium was stained milky white in a concentration-dependent manner at the test initiation in all test item treatment groups. The test medium became clear and colourless in the 50 and 100 mg/L test item treatment groups at the end of the exposure period but a slightly milky white colour was still observed in the 25 mg/L test item treatment group. Precipitates were observed in all of the exposure groups after 24 and 48 hours of exposure in a concentration-dependent manner.

Table 136: Measured concentrations of CM-0237 in the exposure solutions during the 48 h exposure of *Daphnia magna*

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	48 h	Mean	% of nominal
Suspended fluid				
0	< LOQ	< LOQ	n.a.	n.a.
25	20.8	5.18	11.2	44.9
50	45.6	1.83	13.6	27.2
100	85.0	1.68	21.2	21.2
Dissolved fluid				
0	< LOQ	< LOQ	n.a.	n.a.
25	0.304	0.242	0.272	1.09
50	0.289	0.222	0.254	0.508
100	0.296	0.219	0.256	0.256

LOQ = limit of quantification. i.e. 0.1 mg/L

n.a. = not applicable

Biological results

In the latest test with the reference substance, the EC_{50} value determined in three tests was 0.66 – 1.3 mg/L.

Table 137: Immobilisation of *Daphnia magna* exposed to CM-0237 for 48 h in a static test

Nominal concentration [mg/L]	**Cumulative number of immobilised daphnids**	
	After 24 hours	**After 48 hours**
0	0	0
25	0	5
50	0	5
100	0	9

The rate of immobilisation was 45 % in the 100 mg/L test item treatment group after 48 hours of exposure. Thus, the EC_{50} was estimated to be > 21.2 mg/L based on suspended fluid (mean measured concentration) and >0.256 mg/L based on dissolved fluid (mean measured concentration).

RMS comments:

The study was well performed and reported. The study was evaluated against the validity criteria of OECD 202:

– less than 10% immobilization in the control

– dissolved oxygen concentration > 60% of saturation

These validity criteria are fulfilled.

The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. The dissolved concentrations were practically the same at all treatment levels. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore the RMS propose that the geomean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC_{50} of >0.256 mg/L.

Reference:	**Kley, A., Wydra. V. (2012):** Acute Toxicity of 4-OH-TM to Larvae of *Chironomus riparius* in a Static 48-hour Immobilisation Test
Report number:	RD-02402. 67762251 (824-001)
Guideline:	OECD TG 235 (2011)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	4-OH-TM
Lot/Batch No:	31-10193-N.UMEDA
Purity:	95.3%
Species:	Larvae of *Chironomus riparius*, 2 days old.
Treatments:	0.51, 1.9, 7.1, 27 and 100 mg/L (nominal).
Controls:	Reconstituted water (Elendt M4)
Replication:	20 larvae per treatment level and control, divided into 4 groups of 5 animals.
Duration:	48 hours
Test conditions:	Static test design. Temperature: 19 – 20°C Dissolved oxygen: 8.3 – 8.9 mg/L pH: 7.7 – 7.9 Photoperiod: 16 h light : 8 h dark Light intensity: 580 – 850 lux
Observations:	Number of immobile organisms were observed after 24 and 48 hours.
Chemical analysis:	At start and end of exposure period by HPLC-UV method. LOQ was 0.5 mg/L. Filtrated and non-filtrated samples were analysed.
Data analysis:	Due to the low effect. EC_{50}, EC_{20} and EC_{10} could not be calculated. NOEC and LOEC determined directly from raw data.

Results:

Analytical results

At the start of the test, a mean of 93 % of the nominal test concentrations was found in the non-filtrated samples. After 48 hours a mean of 64 % of the nominal value was determined. These results were caused by the settlement of the test item during the test in the test media of 27 and 100 mg/L.

In the filtrated samples, a mean of 40 % of the nominal test concentrations was found in the filtrated samples. After 48 hours a mean of 55 % of the nominal value was determined.

Table 138: Measured concentrations of 4-OH-TM in the exposure solutions during the 48 h exposure of *Chironomus riparius*

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	48 h	Mean [1]	% of nominal
Non-filtrated samples				
0	< LOQ	< LOQ	n.a.	n.a.
0.51	0.483	0.389	0.436	86
1.9	1.70	1.51	1.61	85
7.1	6.59	5.70	6.14	87
27	25.5	12.9	19.2	71
100	92.8	48.6	70.7	71
Filtrated samples				
0	< LOQ	< LOQ	n.a.	n.a.
0.51	0.308	0.387	0.347	68
1.9	1.04	1.54	1.29	68
7.1	3.66	5.62	4.64	65
27	10.4	12.0	11.2	41
100	15.3	14.0	14.6	15

LOQ = limit of quantification. i.e. 0.009 mg/L

n.a. = not applicable

[1] mean value of all replicate measurements after 0 and 48 h

All chemical and physical parameters (dissolved oxygen concentration. pH. and temperature) in the definitive test were within expected ranges.

Biological results

After 48 hours of exposure, no immobilisation was observed in the control and in the test item treatment groups up to and including 7.1 mg/L (nominal concentration). In the test item treatment groups of

nominal 27 and 100 mg/L, an immobilisation of 5 and 10 %, respectively, was observed after 48 hours of exposure.

Table 139: Immobilisation of *Chironomus riparius* exposed to 4-OH-TM for 48 h in a static test

Mean measured concentration [mg/L]	**Cumulative number (%) of immobilised larvae**	
	After 24 hours	After 48 hours
0	0 (0)	0 (0)
0.439	0 (0)	0 (0)
1.62	0 (0)	0 (0)
6.18	0 (0)	0 (0)
19.2	0 (0)	1 (5)
71	0 (0)	2 (10)

Since the concentrations tested did not result in ≥ 50 % immobilization, the 48 h EC_{50} value for *Chironomus riparius* exposed to 4-OH-TM was empirically estimated to be greater than the highest test concentration (> 71 mg/L, mean measured concentration when based on non-filtrated samples).

RMS comments:

The study was evaluated against the validity criteria of OECD 235:

-Less than 15% immobilisation or other signs of toxicity in the control at the end of the test.

-Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

Both these criteria were met and the study is considered as valid. The measured test concentrations in filtrated samples were generally lower than the corresponding values for non-filtrated samples, probably due to low solubility in water. It can be assumed that un-dissolved test substance is less bioavailable for uptake via water, and therefore as a worst case the RMS propose that the mean measured concentrations from the filtrated samples is used for the derivation of ecotoxicology endpoint in this study, resulting in a 48 hour EC_{50} of >14.6 mg/L.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Reference:	**Saito, S. (2002):** Thiophanate-methyl - Inhibition of growth to the alga *Pseudokirchneriella subcapitata*
Report number:	NCAS 02-160. RD-II 02438 (823-003)
Guideline:	OECD TG 201 (1984)
GLP:	Yes
Previous evaluation:	In Addendum 7 to the DAR, 2003
Material and methods:	

Test material:	Thiophanate-methyl technical
Lot/Batch No:	TBC- 035G
Purity:	98.2%
Species:	The green alga *Pseudokirchneriella subcapitata*, initial cell volume of 10000 cells/mL
Test media:	OECD medium, pH 7.8-9.8
Treatments:	1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg/L (nominal)
Controls:	Untreated and solvent control (acetone)
Replication:	Three test vessels containing a volume of 100 ml of water were used per concentration, control and solvent control.
Duration:	96 hours
Test conditions:	Temperature ca 24°C, fluorescent light of 4000-4500 lux continuous illumination
Observations:	Cells concentrations in each solution were determined at 0, 24, 48, 72 and 96 hours of exposure.
Chemical analyses:	The concentrations of Thiophanate-methyl in the test solutions was determined at test start and end.
Data analysis:	Probit analysis for EC_{50} and Dunnet's test for NOEC.

Results:

The mean measured concentrations of dissolved Thiophanate-methyl ranged from 25 to 73 % of the nominal values.

Table 140: Summary of measured dissolved concentrations of Thiophanate-methyl in the test solutions.

Nominal test conc. (mg/L)	0 hours	96 hours	Mean (geomean)	% of nominal
1.56	1.38	0.386	0.780 (0.730)	50 (47)
3.13	2.78	1.33	1.97 (1.92)	63 (61)
6.25	4.93	3.87	4.38 (4.37)	70 (70)
12.5	10.4	7.95	9.12 (9.09)	73 (73)
25	14.6	13.7	14.1 (14.1)	56 (56)
50	21.5	19.9	20.7 (20.7)	41 (41)
125	24.8	26.0	25.4 (25.4)	25 (25)

Based on the arithmetric mean measured test concentrations, it was concluded that the E_bC_{50} values at 72 and 96 hours were 12 (95%CL 10-13) and 13 (95%CL 12-15) mg/L, respectively, and E_rC_{50} values at 24-48, 24-72 and 24-96 hours were >25.4 mg/L. NOEC was determined to be 4.38 for biomass and 9.12 mg/L after 72 hours.

RMS comments:

The study was conducted according to the OECD TG 201 from 1984. In 2006 and 2011, the OECD TG 201 was revised, including additional validity criteria and the additional endpoint of yield. Therefore, the study was re-evaluated by Wirzinger (2015, see below) in order to see whether the study is still valid and complies with current guideline requirements with regard to other parameters (e.g. test conditions, cell number and pH values).

Reference:	**Wirzinger, G. (2015):** Effects of Thiophanate-methyl on the growth of green algae (*Pseudokirchneriella subcapitata*) - Re-calculation of toxicity endpoints of the algal growth study by Saito (2002. Doc. No. 823-003)
Report number:	RD-03363. PP146-00061 (882-007)
Guideline:	OECD TG 201 (2011)
GLP:	No
Previous evaluation:	No, submitted for the purpose of renewal.

Summary:

-Specific growth rate >0.92 day^{-1}, or actual increase >16 times in 72 hours. Actual increase was 92.3 times.

-Mean coefficient of variation for section-by-section specific growth rates in the control cultures < 35%. Actual value was reported to be 33.9%.

-Coefficient of variation of average specific growth rates during the whole test period in replicate control cultures < 7% (for *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*). Actual value was reported to be 1.8%.

According to the current guideline, the pH of the control should not increase by more than 1.5 units during the test. In the study, the pH increased by more than 1.5 units at the end of the test in both controls and in the test item treatment groups up to and including 9.12 mg a.s./L (actual increase 1.6 to 1.9 units). According to the study director, this was due to the photosynthesis by the algae and had no impact on the assessment of the test substance.

Algal growth in terms of yield showed a decrease of more than 50 % compared to the solvent control at the three highest test item treatment groups after 72 hours (see table below).

Table 141: Yield of *Pseudokirchneriella subcapitata* after exposure to Thiophanate-methyl

Mean measured concentration [mg a.s./L]	**Yield [x 10^4 cells/mL] (percent inhibition) after**		
	24 h	**48 h**	**72 h**
Solvent control [1]	2.78	24.8	90.9
0.780	2.75 (1.1) *	25.6 (-3.4) *	96.5 (-6.2)
1.97	2.16 (22.2) *	21.3 (13.8) *	91.3 (-0.5)
4.38	1.68 (39.6) *	17.7 (28.4) *	83.6 (8.0)
9.12	1.07 (61.6) *	11.3 (54.4) *	49.9 (45.1) **

14.1	1.64 (41.1) *	11.1 (55.2) *	35.4 (61.0) **
20.7	-2.46 (189) *	3.57 (85.6) *	27.5 (69.8) **
25.4	0.823 (70.4) *	5.79 (76.6) *	25.1 (72.4) **

Values in parentheses represent percent inhibition compared to solvent control; negative values indicate a higher growth rate in treatment group compared to solvent control

[1] Inhibition of yield was compared to the solvent control as there were not statistically significant differences between the water control and the solvent control

** statistically significantly different compared to solvent control (p ≤ 0.05, Bonferroni-Holm U-test)*

*** statistically significantly different compared to solvent control (p ≤ 0.05, Williams t-test)*

Algal growth results are summarised in the table below. After 72 hours, the maximum growth inhibition relative to the solvent control at the second highest test concentration was 42.6 %.

Table 142: Growth rate of *Pseudokirchneriella subcapitata* after exposure to Thiophanate-methyl

Mean measured concentration [mg a.s./L]	**Growth rate [x 10^4 cells/mL/d] (percent inhibition) after**		
	24 h	48 h	**72 h**
Solvent control [1]	1.32	1.62	1.50
0.780	1.30 (1.6) *	1.63 (-0.5) *	1.52 (-0.9) *
1.97	1.11 (15.6) *	1.53 (5.6) *	1.49 (1.0) *
4.38	0.962 (27.1) *	1.45 (10.6) *	1.47 (2.4) *
9.12	0.684 (48.2) *	1.22 (24.7) *	1.28 (14.7) *
14.1	0.930 (29.5) *	1.21 (25.3) *	1.17 (21.9) *
20.7	-0.151 (112) *	0.604 (62.7) *	0.862 (42.6) *
25.4	0.526 (60.1) *	0.888 (45.1) *	1.03 (31.4) *

Values in parentheses represent percent inhibition compared to control; negative values indicate a higher growth rate in treatment group compared to control

[1] Inhibition of growth rate was compared to the solvent control as there were not statistically significant differences between the water control and the solvent control

** statistically significantly different compared to control (p ≤ 0.05, Bonferroni-Holm U-test)*

The calculated toxicity endpoints for yield and growth rate are summarised in the table below. The E_yC_{10}, E_yC_{20} and E_yC_{50} after 72 hours based on mean measured concentrations were 3.13, 4.93 and 11.8 mg a.s./L, respectively. The E_rC_{10}, E_rC_{20} and E_rC_{50} after 72 hours based on mean measured concentrations were 6.74, 12.1 and 37.2 mg a.s./L, respectively. The NOEC and LOEC for growth rate could not be determined due to mathematical reasons. Based on yield, the NOEC and the LOEC were 4.38 and 9.12 mg a.s./L, respectively.

Table 143: ECx, LOEC and NOEC values for growth rate of *Pseudokirchneriella subcapitata* after 72 hours of exposure to Thiophanate-methyl in a static test system

Growth Function	EC_{10} [mg a.s./L]	EC_{20} [mg a.s./L]	EC_{50} [mg a.s./L]	LOEC [mg a.s./L]	NOEC [mg a.s./L]
Toxicity endpoints after 72 hours					
Yield	3.13 (1.89 – 4.28)	4.93 (3.43 – 6.22)	11.8 (10.1 – 13.6)	9.12	4.38
Growth rate	6.74 (1.00 – 10.5)	12.1 (5.14 – 16.1)	37.2 (25.6 – 169)	n.d.	n.d.

n.d. could not be determined due to mathematical reasons or inappropriate data

RMS comments:

Based on the growth curves from the study, exponential growth in the control and solvent control was limited to the first 72 hours of the test. Therefore, the 72 hour values are considered most relevant for the further assessment. It was noted that the arithmetic mean values were used by the applicant in the re-analysis. In principle, the geometric mean are regarded as more appropriate, however in this case the values were similar. The RMS generally agree with the proposed re-evaluation but has made a check against the validity criteria beside that presented by the applicant.

Table 144: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures. Control and solvent control were pooled in the calculations below.

Replicates	0-72 h				Section by section (day 0-1. 1-2. 2-3)				
	Average growth rate (day^{-1})	St Dev	CV	CV (%)	Average growth rate (day^{-1})	St Dev	CV	Mean CV	Mean CV (%)
A	1.50	0.0256	0.0170	2	1.48	0.56	0.38	0.29	29
B					1.54	0.45	0.29		
C					1.50	0.51	0.34		
D					1.53	0.36	0.24		
E					1.49	0.35	0.23		
F					1.48	0.38	0.25		

In conclusion, the RMS could confirm that the study fulfilled the validity criteria and can be considered as useful for the risk assessment.

Reference:	**Kley, A., Wydra, V. (2012):** Toxicity of Thiophanate-methyl 500 SC to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test
Report number:	RD-02400, 67723210 (823-007)
Guideline:	OECD TG 201 (2006)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal.

Material and methods:	
Test material :	Thiophanate-methyl 500 SC
Lot/Batch #:	0151107
Content of a.s.:	500 g Thiophanate-methyl/L (nominal), 41.0 % w/w (analysed)
Treatments:	Nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg formulation/L.
Control:	Reconstituted water (OECD medium)
Species:	*Pseudokirchneriella subcapitata* Strain 61.81 SAG
Source:	In-house culture
Test unit:	50 mL Erlenmeyer flasks with 50 mL test medium, 5000 algal cells/mL at test start.
Replication:	Three replicate flasks of the test concentrations and six replicates for the control were included in the test. Additionally, one replicate of each test concentration and of the control was prepared without algae to provide a blank for the spectrophotometrical measurements.
Duration:	72 hours
Test conditions:	Static conditions
	Temperature: 21 – 23°C
	pH: 7.8 – 8.0 (test start); 8.1 – 8.6 (test end)
	Photoperiod: continuous uniform illumination
	Light intensity: 7820 lux (7370 – 8240 lux)
Observations:	At each subsequent 24-hour interval, the cell densities were determined by spectrophotometrical measurements. One sample was removed from each flask for counting. Observations of the health of the algal cells and appearance of the test item in the test media were also made and recorded at each 24-hour interval.
Chemical analysis:	The samples of the test media (containing algae) sampled at the start and at the end of the test after 72 hours of exposure were analysed with high performance liquid chromatography with ultraviolet detection (HPLC/UV).
Data analysis:	The inhibition of algal growth was determined from yield and average specific growth rate. Based on the calculated cell densities, the 72-hour E_rC_{50} and E_yC_{50}, the corresponding EC_{10} values and where possible their 95 % confidence limits were calculated by Probit analysis. For the determination of the 72-hour LOEC and NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by the Williams Test, respectively.

Results:

Analytical results

At the start of the test, 96 % of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 74 % of the nominal value was determined (average of all test concentrations). Mean measured concentrations were in the range of 81.3 to 90.0 % of the nominal test concentrations, therefore all endpoints refer to nominal concentrations.

A slight turbidity caused by the test item was observed at the test start in the 32 mg/L test item treatment group. In the 100 mg/L test item treatment group, turbidity of the test medium was observed during the whole test.

Table 145: Measured Thiophanate-methyl concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata* to Topsin M 500 SC

Nominal concentration [mg/L]		**Measured concentration [1] [mg a.s./L]**					
Thiophanate-methyl 500 SC	**Thiophanate-methyl**	**0 hours**	**% of nominal**	**72 hours**	**% of nominal**	**Geomean measured [mg a.s./L]**	**% of nominal**
0 (control)	0 (control)	< LOD [2]	n.a.	< LOD [2]	n.a.	<LOD	n.a.
1.0	0.410	0.394	96	0.273	66.5	0.328	80.0
3.2	1.31	1.24	95	0.900	68.5	1.06	80.9
10	4.10	3.84	93.5	2.84	69.5	3.30	80.4
32	13.1	13.7	104	9.96	76.0	11.7	89.3
100	41.0	36.5	89.0	35.8	87.5	36.1	88.0

[1] Results represent rounded results calculated on the exact analytical data

[2] LOD = limit of detection (7 µg a.s./L) n.a. = not applicable

All chemical and physical parameters (dissolved oxygen concentration, pH, temperature) in the definitive test were within expected ranges.

Biological results

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 177); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.1 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.7 %).

Cell densities determined at each observation interval are presented in the table below. Cells appeared to be normal throughout the exposure period for the control and test item concentrations up to and including 32 mg/L. At a nominal concentration of 100 mg/L, the cells were smaller and showed a round shape compared to the cells of the control.

Table 146: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC

Nominal concentration of Topsin M 500 SC [mg/L]	**Mean Cell Concentration (x 10^4 cells/mL) after**			
	0 h	**24 h**	**48 h**	**72 h**
0 (control)	0.5	2.94 ± 0.411	13.8 ± 1.11	88.5 ± 7.66
1.0	0.5	2.98 ± 0.424	13.1 ± 0.883	77.8 ± 6.18
3.2	0.5	2.41 ± 0.245	13.2 ± 2.36	77.2 ± 5.65
10	0.5	1.84 ± 0.122	14.8 ± 2.36	73.6 ± 7.37
32	0.5	1.14 ± 0.122	11.2 ± 0.490	50.5 ± 1.29
100	0.5	0.500 ± 0.00	8.98 ± 1.05	6.37 ± 4.29

Cell biomass, expressed as yield, is presented in the table below. Based on the results, Williams Test detected a significant reduction in yield in treatment levels ≥ 1.0 mg/L compared to the control data. Therefore, the 72-hour NOEC for yield was determined to be ≤ 1.0 mg a.s./L. The 72-hour E_yC_{50} value was determined to be 31.8 mg/L.

Table 147: Mean cell biomass (expressed as yield) of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC

Nominal concentration of Topsin M 500 SC [mg/L]	**Mean yield (x 10^4 cells/mL) after**			
	24 h	**48 h**	**72 h**	**Inhibition [%] [1]**
0 (control)	2.44 ± 0.411	13.3 ± 1.11	88.0 ± 7.66	-
1.0	2.48 ± 0.424	12.6 ± 0.883	77.3 ± 6.18 *	12.2
3.2	1.91 ± 0.245 *	12.7 ± 2.36	76.7 ± 5.65 *	12.8
10	1.34 ± 0.122 *	14.3 ± 2.36	73.1 ± 7.37 *	16.9
32	0.638 ± 0.122 *	10.7 ± 0.490 *	50.0 ± 1.29 *	43.3
100	0.00 ± 0.00 *	8.48 ± 1.05 *	5.87 ± 4.29 *	93.3

[1] *Percent inhibition after 72 hours compared to control.*

** Significantly reduced compared to the control, based on Williams Test, α =0.05, one-sided*

Growth rates are presented in the table below. Based on the results of the Williams Test a significant reduction in average growth rate in treatment levels 32 mg/L compared to the control data was detected. Therefore, the 72-hour NOEC for average growth rate was determined to be 10 mg/L. The 72-hour E_rC_{50} value was determined to be 88.3 mg/L.

Table 148: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC

Nominal concentration of Topsin M 500 SC [mg/L]	Growth rate ($days^{-1}$) after			
	0 – 24 h	0 – 48 h	0 – 72 h	Inhibition [%] [1]
0 (control)	1.76 ± 0.136	1.66 ± 0.040	1.72 ± 0.029	-
1.0	1.78 ± 0.144	1.63 ± 0.034	1.68 ± 0.027	2.5
3.2	1.57 ± 0.105 *	1.64 ± 0.052	1.68 ± 0.024	2.6
10	1.30 ± 0.068 *	1.69 ± 0.082	1.66 ± 0.034	3.6
32	0.818 ± 0.105 *	1.55 ± 0.022 *	1.54 ± 0.009 *	10.8
100	0.00 ± 0.00 *	1.44 ± 0.057 *	0.758 ± 0.342 *	56.0

[1] *Percent inhibition after 72 hours compared to control.*

** Significantly reduced compared to the control, based on Williams Test, α =0.05, one-sided*

The 72-hour E_yC_{10}, E_yC_{20} and E_yC_{50} and NOEC values determined for this test as well as the 72-hour E_rC_{10}, E_rC_{20} and E_rC_{50} and NOEC values are presented in the table below.

Table 149: Toxicity endpoints for growth rate and yield of *Pseudokirchneriella subcapitata* after exposure to Topsin M 500 SC based on nominal test concentrations.

Parameter	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]	NOEC [mg/L]
0 – 72 h yield (95 % confidence intervals)	8.12 (3.20 – 12.7)	13.0 (6.66 – 18.4)	31.8 (23.4 – 43.3)	≤ 1.0
0 – 72 h growth rate (95 % confidence intervals)	30.2 (13.8 – 42.4)	43.6 (25.7 – 56.0)	88.3 (72.2 – 112.6)	10

RMS comments:

The study was well performed and reported and the validity criteria of OECD TG 201 (2011) were met, except that since the measured test concentrations declined below 80%, the results should have been based on the measured values and not the nominal. The applicant (Wirzinger and Ruhnke, 2016; 882-008) has re-evaluated the endpoints based on the geometric mean measured concentrations of the test item, i.e. 0.328, 1.06, 3.30, 11.7 and 36.1 mg a.s./L. The results of the re-evaluation of the study using ToxRat (version 3.2.1) are presented in the table below.

Table 150: Toxicity endpoints for effects on yield and growth rate of the *Pseudokirchneriella subcapitata* study (Kley & Wydra, 2012) after exposure to Thiophanate-methyl 500 SC

Parameter [1]	**EC_{10} [mg a.s./L]**	**EC_{20} [mg a.s./L]**	**EC_{50} [mg a.s./L]**	**NOEC [mg a.s./L]**
0 – 72 h yield (95 % confidence intervals)	2.7 (2.0 – 3.4)	4.4 (3.5 – 5.2)	11.2 (9.9 – 12.7)	< 0.33
0 – 72 h growth rate (95 % confidence intervals)	10.5 (6.8 – 16.3)	14.6 (10.5 – 20.2)	27.3 (22.4 – 33.2)	3.3

[1] *EC_{10}, EC_{20} and EC_{50} calculated with linear regression using probit analysis*

The results indicate that the formulation (expressed per content of active substance) is of similar toxicity to algae as Thiophanate-methyl alone.

Author: Bell, G.

Title: Caarbendazim techn. – Algal growth inhibition

Date: 31 July 96

Doc ID: SNG 44(a)/960463; BVL No. 1843621

Guidelines: OECD 201

GLP: Yes

Validity: Yes

Aim of the study was to asses the toxicity of carbendazim to the algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a static test.

Methods

Test substance: Carbendazim tech, 99.5% purity

Test concentrations:

0.46, 1.0, 2.2, 4.6 und 10 mg carbendazim/L

The solubility limit of carbendazim in the test medium was 10 mg/L. Carbendazim was applied directly to the medium then sonicated and stirred with magnetic bars for 24 h.

Results

The measured test concentrations were:

0.51, 1.1, 2.5, 4.9 und 11 mg carbendazim/L, respectively (recovery 106 – 113 %)

Table 151: Toxicity of carbendazim to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

Endpoint	Value (mg/L)	95 % confidence interval (mg/L)	Time (d)	ai	n/r/i
NOECbiomass	2.5		3		r
EbC50	7.7	6.6 – 8.9	3		r
ErC50	>11		5		r

n = nominal; r = real; i = initial

Conclusion

Report results listed and evaluated in the Addendum 2 to the monograph (26.01.2000).

For *Pseudokirchneriella subcapitata* exposed to carbendazim in a static test, the NOEC (72 h) was 2.5 mg/L, the EbC_{50} (72 h) = 7.7 mg/L and the ErC_{50} >11 mg/L.

Reference:	**Baba, K. (2012): 4-OH-TM:** Growth Inhibition Study in *Pseudokirchneriella subcapitata*
Report number:	RD-02399. NCAS 11-177 (823-006)
Guideline:	OECD TG 201 (2011)
GLP:	Yes.
Previous evaluation:	No. submitted for the purpose of renewal.
Material and methods:	
Test material:	4-OH-TM (Thiophanate-methyl metabolite)
Lot/Batch No:	31-5267-DT
Purity:	99.2%
Species:	The freshwater green alga *Pseudokirchneriella subcapitata* (Strain ATCC 22662), cell density 5000 cells per mL.
Test media:	OECD medium
Treatments:	0.294, 0.960, 3.06, 9.80, 31.4 and 100 mg/L (nominal)
Controls:	Untreated
Replication:	Three replicate flasks of the test concentrations and six replicates for the control were included in the test.
Duration:	72 hours
Test conditions:	Temperature: 23 ± 2 °C, pH 8.0 – 8.1, continuous uniform illumination. Light intensity was 63 - 82 µE/m²/s
Observations:	At each subsequent 24-hour interval, cell counts were conducted on the replicate vessels using an electronic particle counter or a microscope with a counting chamber. Observations of the health of the algal cells and on the appearance of the test media were also made and recorded at each 24-hour interval.
Chemical analyses:	At test initiation (0 hour) and test termination (72 hours), a single sample was removed from each test concentration and the control and analysed for

	4-OH-TM with HPLC. For the determination of 4-OH-TM in dissolved fluids, an aliquot of the test medium was collected and centrifuged. The supernatant fluid was filtrated, mixed with methanol in the same volume and analysed with HPLC.
Data analysis:	The growth curves were prepared by plotting the time of measurement against the mean measured cell density. The average specific growth rate for a specific period is calculated as the logarithmic increase in biomass. Since the per cent inhibition of the growth rate was less than 50 % even at the highest concentration tested, the 72 h E_rC_{50} was not calculated statistically. The No-Observed-Effect Concentration (NOEC) is the highest test concentration which demonstrated no statistically adverse effect on growth. The data were first checked for homogeneity of variance using Bartlett's Test. If the data sets passed the test, then 1-way ANOVA and Dunnett's test were used to determine the NOEC.

Results:

Analytical results

At the start of the exposure period, the control and the 0.294, 0.960, 3.06 and 9.80 mg/L treatment groups were clear and colourless with no precipitate or undissolved particles present, whereas the test medium of the 31.4 and 100 mg/L test item treatment groups was a white, cloudy dispersion with precipitates and floating particles attached to the upper part of the test vessels. After 48 and 72 hours a small amount of floating particles attached to the upper part of the test vessels was observed in the 9.80 mg/L test item treatment group. After 72 hours, the colour of the test medium in the 31.4 and 100 mg/L test item treatment groups became clearer and no precipitate was present, but the amount of floating particles increased.

The geomean measured concentrations of the suspended fluids and of the dissolved fluids are given in the table below. The endpoints were based on the mean measured concentrations of dissolved fluids (from centrifuged samples).

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

Table 152: Measured 4-OH-TM concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata*

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	**72 h**	**Geomean**	**% of nominal**
Suspended fluid				
0	< LOD	< LOD	n.a.	n.a.
0.294	0.311	0.190	0.246	83.7
0.960	0.875	0.688	0.778	81.0
3.06	2.82	2.48	2.65	86.6
9.80	9.56	8.32	8.93	91.1

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	**72 h**	**Geomean**	**% of nominal**
31.4	30.4	22.3	26.1	83.1
100	104	51.5	74.7	74.7
Dissolved fluid				
0	< LOD	< LOD	n.a.	n.a.
0.294	0.296	0.186	0.237	80.6
0.960	0.856	0.681	0.765	79.7
3.06	2.75	2.43	2.59	84.6
9.80	8.32	8.10	8.21	83.8
31.4	13.0	13.7	13.3	42.4
100	14.6	15.5	15.0	15.0

LOD = limit of detection, i.e. 0.050 mg/L
n.a. = not applicable

Biological results

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 200); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.5 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.44 %).

Cell densities and growth rates determined at each observation interval are presented in the tables below. There were no morphological abnormalities in any of the treatment groups after 72 hours of exposure.

Table 153: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to 4-OH-TM

Mean measured concentration [1] [mg/L]	**Mean Cell Concentration [x 10^4 cells/mL] after**			
	0 h	**24 h**	**48 h**	**72 h**
0 (control)	0.5	2.45 ± 0.251	16.3 ± 1.45	99.8 ± 7.37
0.237	0.5	2.53 ± 0.176	18.3 ± 0.208	121 ± 3.79
0.765	0.5	2.86 ± 0.283	17.4 ± 0.611	112 ± 2.08
2.59	0.5	2.89 ± 0.191	19.0 ± 0.889	109 ± 4.16
8.21	0.5	2.74 ± 0.325	15.5 ± 0.116	92.2 ± 3.19
13.3	0.5	2.50 ± 0.346	18.4 ± 0.346	94.7 ± 2.89
15.0	0.5	2.77 ± 0.404	18.3 ± 1.70	96.7 ± 13.3

[1] *Based on dissolved fluids*

Table 154: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to 4-OH-TM

Mean measured concentration [1] [mg/L]	**Growth rate ($days^{-1}$) after**				
	0 – 24 h	**24 – 48 h**	**48 – 72 h**	**0 – 72 h**	**Inhibition [%] [2]**
Control	0.066 ± 0.004	0.079 ± 0.006	0.076 ± 0.004	0.074 ± 0.001	-
0.237	0.067 ± 0.003	0.083 ± 0.003	0.079 ± 0.001	0.076 ± 0.0004 *	-3.67
0.765	0.073 ± 0.004	0.075 ± 0.004	0.078 ± 0.001	0.075 ± 0.0003	-2.31
2.59	0.073 ± 0.003	0.079 ± 0.001	0.073 ± 0.001	0.075 ± 0.001	-1.81
8.21	0.071 ± 0.005	0.072 ± 0.005	0.074 ± 0.002	0.073 ± 0.001	1.41
13.3	0.067 ± 0.006	0.083 ± 0.007	0.068 ± 0.002	0.073 ± 0.0004	0.904
15.0	0.071 ± 0.006	0.079 ± 0.002	0.069 ± 0.009	0.073 ± 0.002	0.590

[1] *Based on dissolved fluids*

[2] *Per cent inhibition compared to control.*

** Significantly different compared to the control, based on Dunnett's Test.*

Since the per cent inhibition based on mean measured concentrations of the dissolved fluids was less than 50 % even at the highest test item concentration, the E_rC_{50} was determined to be > 15.0 mg/L. Based on the results of Dunnett's test, there was a significant difference in growth compared to the control in the test item treatment group of 0.237 mg/L. As the growth rate in this test item treatment group was higher than in the control, this was not considered to be treatment-related. Therefore, the 72-hour NOEC for growth rate was determined to be 15.0 mg/L.

In conclusion, based on mean measured concentrations of dissolved fluids the 72-hour EC_{50} value for growth rate (E_rC_{50}) of *Pseudokirchneriella subcapitata* exposed to 4-OH-TM was determined to be >15.0 mg/L. The 72 h NOEC value for growth rate ($NOEC_r$) was calculated to be 15.0 mg/L based on mean measured values of dissolved fluids.

The EC_{10} and EC_{20} values were not reported. However, the E_rC_{50} is the relevant endpoint for the risk assessment for algae.

RMS comments:

The study was well performed and reported. The RMS agree with the validity assessment provided by the applicant, and that the results should be based on the mean measured dissolved test concentrations. An overview of the validity check is given in the table below.

Table 155: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures.

Replicates	0-72 h				Section by section (day 0-1. 1-2. 2-3)				
	Average growth rate (day^{-1})	St Dev	CV	CV (%)	Average growth rate (day^{-1})	St Dev	CV	Mean CV	Mean CV (%)
A	1.76	0.025	0.014	1.40	1.79	0.09	0.05	0.105	11
B					1.75	0.19	0.11		
C					1.75	0.35	0.20		
D					1.73	0.18	0.10		
E					1.79	0.11	0.06		
F					1.78	0.20	0.11		

Reference:	**Baba, K. (2012):** CM-0237: Growth Inhibition Study in *Pseudokirchneriella subcapitata*
Company Report No.:	RD-02398, NCAS 11-176 (823-005)
Guideline:	OECD TG 201 (2011)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal
Material and methods:	
Test material:	CM-0237 (Thiophanate-methyl metabolite)
Lot/Batch No:	31-02122-I.TAKEUCHI
Purity:	98.1%
Species:	*Pseudokirchneriella subcapitata (*Strain ATCC 22662), initial cell density 5000 cells/mL
Test media:	OECD medium
Treatments:	0.150, 0.300, 0.600, 1.20 and 2.4 mg/L (nominal)
Controls:	Untreated
Replication:	Three replicate flasks of the test concentrations and six replicates for the control were included in the test.
Duration:	72 hours
Test conditions:	Temperature: 23.0-23.3°C, pH 7.9 – 8.0, continuous uniform illumination.
	Light intensity was 70 - 82 µE/m²/s
Observations:	At each subsequent 24-hour interval, cell counts were conducted on the replicate vessels using an electronic particle counter or a microscope with a counting chamber. Observations of the health of the algal cells and on the

	appearance of the test media were also made and recorded at each 24-hour interval.
Chemical analyses:	At test initiation (0 hour) and test termination (72 hours), a single sample was removed from each test concentration and the control and analysed for CM-0237. For the determination of CM-0237 in suspended fluids, an aliquot of the test medium was collected and mixed with methanol. The mixture was centrifuged and the supernatant fluid was analysed with HPLC. For the determination of CM-0237 in dissolved fluids, an aliquot of the test medium was collected and centrifuged. The supernatant fluid was filtrated, mixed with methanol in the same volume and analysed with HPLC.
Data analysis:	The growth curves were prepared by plotting the time of measurement against the mean measured cell density. The average specific growth rate for a specific period is calculated as the logarithmic increase in biomass. Since the per cent inhibition of the growth rate was less than 50% even at the highest concentration tested, the 72 h ErC_{50} was not calculated statistically. The No-Observed-Effect Concentration (NOEC) is the highest test concentration which demonstrated no statistically adverse effect on growth. The data were first checked for homogeneity of variance using Bartlett's Test. If the data sets passed the test, then 1-way ANOVA and Dunnett test were used to determine the NOEC. All statistical determinations were performed by using the Yukms software Statlight 2000.

Results:

Analytical Results

During the exposure period, the control and the 0.150, 0.300, 0.600 and 1.20 mg/L test item treatment groups were clear and colourless. The test medium of the 2.40 mg/L test item treatment group was white with cloudy dispersions attributed to the test substance at test initiation and after 24 hours. After 24 hours, a small amount of floating particles attached to the upper part of the test vessels were observed in all exposure groups.

The mean measured concentrations of the suspended fluids and of the dissolved fluids are presented in the table below.

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

Table 156: Measured CM-0237 concentrations in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata*

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	**72 h**	**Mean**	**% of nominal**
Suspended fluid				
0	< LOD	< LOD	n.a.	n.a.
0.150	0.110	0.091	0.100	66.7

Nominal concentration [mg/L]	**Measured concentration [mg/L]**			
	0 h	**72 h**	**Mean**	**% of nominal**
0.300	0.212	0.143	0.175	58.3
0.600	0.427	0.211	0.306	51.0
1.20	0.893	0.325	0.562	46.8
2.40	1.71	0.411	0.911	38.0
Dissolved fluid				
0	< LOD	< LOD	n.a.	n.a.
0.150	0.0596	0.107	0.081	54.0
0.300	0.104	0.128	0.116	38.7
0.600	0.141	0.153	0.147	24.5
1.20	0.154	0.171	0.162	13.5
2.40	0.185	0.179	0.182	7.58

LOD = limit of detection, i.e. 0.050 mg/L

n.a. = not applicable

Biological results

The study was considered to be valid as the following validity criteria were met: the cell growth in the control must increase from the initial density by more than 16 times after 72 hours of growth (actual increase 201); the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35 % (actual: 10.7 %). The CV for the average growth rate of the control for the entire test period (0- to 72-hour growth rate) should not exceed 7 % (actual 1.82 %).

Cell densities determined at each observation interval, and corresponding growth rates are presented in the tables below.

Table 157: Mean cell density of *Pseudokirchneriella subcapitata* after exposure to CM-0237

Mean measured concentration [1] [mg/L]	**Mean Cell Concentration [x 10^4 cells/mL] after**			
	0 h	**24 h**	**48 h**	**72 h**
Control	0.5	2.44 ± 0.367	15.9 ± 1.59	101 ± 9.46
0.081	0.5	2.7 ± 0.380	18.3 ± 1.36	126 ± 15.4
0.116	0.5	2.48 ± 0.032	16.5 ± 1.21	123 ± 14.0
0.147	0.5	2.64 ± 0.334	20.0 ± 1.97	131 ± 13.6
0.162	0.5	2.70 ± 0.557	18.1 ± 1.62	101 ± 9.54
0.182	0.5	2.67 ± 0.551	20.5 ± 3.93	108 ± 15.0

[1] *Based on dissolved fluids*

Table 158: Mean growth rate of *Pseudokirchneriella subcapitata* after exposure to CM-0237

Mean measured concentration [1] [mg/L]	**Growth rate ($days^{-1}$) after**				
	0 – 24 h	**24 – 48 h**	**48 – 72 h**	**0 – 72 h**	**Inhibition [%] [2]**
Control	0.066 ± 0.006	0.078 ± 0.007	0.077 ± 0.004	0.074 ± 0.001	-
0.081	0.070 ± 0.006	0.080 ± 0.006	0.080 ± 0.002	0.077 ± 0.002 *	-4.30
0.116	0.067 ± 0.001	0.079 ± 0.003	0.084 ± 0.004	0.077 ± 0.002	-3.90
0.147	0.069 ± 0.005	0.085 ± 0.001	0.078 ± 0.003	0.077 ± 0.001 *	-4.94
0.162	0.070 ± 0.009	0.080 ± 0.012	0.072 ± 0.006	0.074 ± 0.001	-0.135
0.182	0.069 ± 0.009	0.085 ± 0.016	0.070 ± 0.007	0.075 ± 0.002	-1.36

[1] *Based on dissolved fluids*

[2] *Per cent inhibition compared to control.*

** Significantly different compared to the control, based on Dunnett's Test.*

Cells in the control were observed to be normal throughout the exposure period. Swelled cells were observed in the 0.150, 0.300 and 0.600 mg/L treatment groups, whereas atrophied cells were observed in the 1.2 and 2.4 mg/L treatment groups. Since the per cent inhibition based on mean measured concentrations of the dissolved fluids was less than 50 % even at the highest test item concentration, the E_rC_{50} was determined to be > 0.182 mg/L.

Based on the results of Dunnett's test, there was a significant difference in growth compared to the control in the test item treatment groups of 0.150 and 1.2 mg/L. As the growth rates in these test item treatment groups were higher than in the control, this was not considered to be treatment-related. Therefore, the 72-hour NOEC for growth rate ($NOEC_r$) was determined to be 0.182 mg/L.

In conclusion, based on mean measured concentrations of dissolved fluids the 72-hour EC_{50} value for growth rate (E_rC_{50}) of *Pseudokirchneriella subcapitata* exposed to CM-0237 was determined to be > 0.182 mg/L. The 72 h NOEC value for growth rate ($NOEC_r$) was calculated to be 0.182 mg/L based on mean measured values of dissolved fluids.

The EC_{10} and EC_{20} values were not reported. However, the E_rC_{50} is the relevant endpoint for the risk assessment for algae.

RMS comments:

The study was well performed and reported. The RMS agree with the validity assessment provided by the applicant, and that the results should be based on the mean measured dissolved test concentrations. It is proposed, though, that the NOEC is set to <0.081 mg/L, due to the observed effects (swelled/atrophied cells)

at all treatment levels. However, this will have no impact on the risk assessment. An overview of the validity check is given in the table below.

Table 159: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures.

Replicates	0-72 h				Section by section (day 0-1. 1-2. 2-3)				
	Average growth rate (day^{-1})	**St Dev**	**CV**	**CV (%)**	**Average growth rate (day^{-1})**	**St Dev**	**CV**	**Mean CV**	**Mean CV (%)**
A	1.77	0.032	0.018	1.83	1.76	0.28	0.16	0.107	11
B					1.77	0.31	0.17		
C					1.76	0.31	0.18		
D					1.71	0.05	0.03		
E					1.79	0.13	0.08		
F					1.81	0.05	0.03		

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Not relevant, no data available.

11.6 Long-term aquatic hazard

The available and reliable data on chronic toxicity of thiophanate-methyl, its major environmental metabolite carbendazim and additional metabolites to aquatic organisms is summarised in the table below.

Table 160: Summary of relevant information on chronic aquatic toxicity. Key data are marked in bold text.

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Laboratory tests					
Fish					
Danio rerio	Thiophanate-methyl	35 days, ELS, flow-through	NOEC EC_{10}	not reported **0.39** (mm)	Report number 826-002 (2014)
Danio rerio	Carbendazim	35 days, ELS, flow-through	NOEC EC_{10}	Not reported	Report number 826-002 (2014)
Oncorhynchus mykiss	Carbendazim*	21 days, flow-through	NOEC	**0.0032** (nom)	Report number A40788, cited in EFSA (2010) *
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.16** (mm) Not reported Not reported	Handley, J.W. et al. (1990) ; Wirzinger and Ruhnke (2016)
Daphnia magna	TOPSIN M WDG (Thiophanate-methyl)	21 d, aged test item*	NOEC EC_{10}	0.0373 (mm) 0.0285 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Daphnia magna	TOPSIN M WDG (Carbendazim)	21 d, aged test item*	NOEC EC_{10}	0.0177 (mm) 0.0149 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)
Daphnia magna	Carbendazim*	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.0015** (nom) Not reported Not reported	Kelly et al. (1997), cited in EFSA (2010) *
Sediment-dwelling organisms					
Chironomus riparius	Thiophanate-methyl	28 d, water spiked	NOEC EC_{10} EC_{20}	0.44 (init. meas.) Not reported Not reported	Memmert. U. (2002)
Chironomus riparius	Carbendazim*	28 d, water spiked	NOEC EC_{10} EC_{20}	0.0133 (nom) Not reported Not reported	Sowig & Gosch (2002), cited in EFSA (2010) *
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	NOE_rC	n.d. (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	NOE_rC	3.28 (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	NOEC	Not reported	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	NOEC	15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	NOEC	0.182 (mm, dissolved)	Baba, K. (2012)

* Refer to the EFSA conclusion on the peer review of the active substance Carbendazim, EFSA (2010). The study summaries provided below for Carbendazim are derived from the Draft Re-Assessment Report produced by Germany in their re-evaluation of the substance under Regulation 1107/2009. Note that these studies were not evaluated by Sweden at this stage.

11.6.1 Chronic toxicity to fish

Reference:	**xxxx (2014):** Zebrafish (*Danio rerio*), Early Life Stage Toxicity Test, Flow through conditions - Test item: Thiophanate-methyl
Report number:	RD-02818, NIS-002/4-43/A (826-002)
Guideline:	OECD TG 210 (1992)
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal.
Material and methods:	
Test material:	Thiophanate-methyl
Lot/Batch No:	TBC-035G
Purity:	97.9 %
Species:	Zebrafish (*Danio rerio*). Freshly fertilised eggs, less than 4 hours old.
Treatments:	The nominal test concentrations of Thiophanate-methyl were 125, 250,

	500, 1000 and 2000 µg a.s./L.
Controls:	Purified drinking water
Replication:	Four replicates per test level, 60 eggs per test level, 15 eggs per test vessel.
Duration:	35 days
Test conditions:	Flow-through. Each group of 15 eggs was placed into a fry chamber suspended in each test vessel. After hatching, the larvae were fed daily ad libitum with newly hatched brine shrimp nauplii (Artemia salina).Temperature: 24.3 – 25.7°C Dissolved oxygen: 93 – 103 % of saturation pH: 7.9 – 8.4 Hardness: 1.1 – 1.2 mg/L Photoperiod: Light/dark cycle of 12 hours/12 hours
Observations:	Qualitative observations on hatching and survival were made daily. Dead embryos, larvae and juvenile fish were removed as soon as observed. Observations on abnormal appearance of behaviour were made daily, too. After 14, 21, 28 and 35 days post fertilisation (pf), the fish larvae were digitally photographed. Post hatch survival rates were estimated by evaluating photographs using electronically supported counting and analysis. At test end, fish lengths and group dry weights of replicate fish groups were measured. Oxygen concentration and pH were measured in each test vessel directly before adding the eggs and at least twice a week thereafter. The water temperature was measured each working day in all test vessels. Additionally, the water temperature was continuously measured in two control test vessels.
Chemical analysis:	LC-MS/MS. LOQ was 50 µg/L for thiophanate-methyl and 1.0 µg/L for MBC (carbendazim).
Data analysis:	The evaluation of the effect concentrations was based on measured test item concentrations of Thiophanate-methyl. For each endpoint the arithmetic mean of all replicates (n=4) was calculated per dose level and control. To confirm normal distribution, Shapiro Wilk's test was performed with each data set. To detect homogeneity of variances, Levene's test was performed. The data were analysed for statistical differences as compared with the untreated control by performing ANOVA, followed by Williams' test to calculate NOECs. William's test, as a trend based test, was chosen, since monotonous dose responses were observed or biologically expected for all endpoints. All data sets were tested against the control. Beside the NOEC determination, a probit analysis was performed to determine EC_{10} and EC_{20}.

Results:

All validity criteria for controls mentioned by the OECD guideline were met: The dissolved oxygen concentration was between 60 and 100 % throughout the test; the water temperature did not differ by more than ± 1.5 °C between successive days at any time during the test and was kept within 25 °C ± 2 °C the survival rate (post hatch) in the controls was greater than 70 %.

Analytical results

The chemical analyses of the test media revealed mean concentrations for the single test vessels between 81 and 123 % of the nominal concentrations. The effect data evaluation was based on the mean measured concentrations. The mean measured test concentrations were between 93.5 % and 103 % of the nominal values. Thus, the treatment levels were defined as 123, 256, 509, 939 and 1870 µg a.s./L.

In addition to the chemical analysis of Thiophanate-methyl the concentrations of Carbendazim in the different treatments were also measured. For Carbendazim the mean concentrations per treatment were found to be between 6.3 and 21.0 % of the Carbendazim equivalents of the nominal concentrations of Thiophanate-methyl (the nominal concentrations of Carbendazim were calculated using the molar weight quotient of Carbendazim : Thiophanate methyl of 0.55838) and were calculated to be 14.6, 20.9, 28.1, 44.2 and 69.9 µg Carbendazim/L.

Table 161: Measured Thiophanate-methyl and Carbendazim concentrations in the exposure solutions during the exposure of zebrafish

Nominal concentration [µg/L]		**Mean measured concentration**			
		Thiophanate-methyl		**Carbendazim**	
Thiophanate-methyl	**Carbendazim***	µg/L	% of nominal	µg/L	% of nominal
0 (control)	0 (control)	> LOQ	-	> LOQ	-
125	70	123 ± 9.1	98.1 ± 7.2	14.6 ± 0.3	21.0 ± 0.5
250	140	256 ± 6.4	103 ± 2.6	20.9 ± 0.6	15.0 ± 0.4
500	279	509 ± 38.9	102 ± 7.8	28.1 ± 3.3	10.0 ± 1.2
1000	558	939 ± 44.5	93.9 ± 4.4	44.2 ± 2.5	7.9 ± 0.4
2000	1117	1870 ± 36.0	93.5 ± 1.8	69.9 ± 4.2	6.3 ± 0.4

LOQ of Thiophanate-methyl: 50 µg/L, LOQ for Carbendazim: 1 µg/L

** Carbendazim equivalents of the nominal concentrations of Thiophanate-methyl (the nominal concentrations of Carbendazim were calculated using the molar weight quotient of Carbendazim : Thiophanate methyl of 0.55838)*

All chemical and physical parameters in the test were within expected ranges. The oxygen saturation was between 93 and 103 %. The water temperature was measured to be in a range of 24.3 to 25.7 °C throughout the study. The pH in the test vessels was between 7.9 and 8.4. The test media was of clear appearance throughout the whole study.

Biological results

Hatch of the fish larvae was not affected by the test item. The hatching success was 100 % at all test concentrations of Thiophanate-methyl (NOEC ≥ 1870 µg a.s./L). The evaluation of post hatch survival revealed a significant decrease at 939 and 1870 µg a.s./L after 28 and 35 days (test end). Statistically significant reduced post hatch survival at the test item treatment groups of 256 and 509 µg a.s./L was observed after 14 and 21 days pf. The respective rates of post hatch survival thereafter were almost comparable to those of after 21 days pf, but no statistically significant differences to the control were found at all later observation dates. Based on this observation, the decreased survival at 256 and 509 µg a.s./L is not considered as biologically relevant. The EC_{10} for this endpoint was calculated to be 390 µg a.s./L (95 % confidence intervals 147 – 645 µg a.s./L) and the EC_{20} was determined to be 1279 µg a.s./L (95 % confidence intervals 769 – 3877 µg a.s./L).

Fish lengths were recorded on day 35 (test end). The single dry weights were calculated by dividing the group dry weights by the number of remaining fish on day 35 pf. Fish lengths and the single dry weights were found to be not significantly inhibited by Thiophanate-methyl (NOEC ≥ 1870 µg a.s./L). An increase of both lengths and weights was observed at all treatments of Thiophanate-methyl, which was not concentration dependent. The single dry weight was between 1.7 to 2.0 times higher than the single dry weight of the control. The observed increase in growth was not considered by the applicant as negative effect on the fish. No abnormality in the fish behaviour was observed.

A summary of egg hatch, post hatch survival, length and wet weight is presented in the table below.

Table 162: Summary of hatching, post hatch survival, length and wet weight of early life stages of zebrafish following exposure to Thiophanate-methyl under flow through conditions

Mean measured concentration [µg a.s./L]	**0 (control)**	**123**	**256**	**509**	**939**	**1870**
Number of introduced eggs (n)	60	60	60	60	60	60
Embryo hatching success [%]	100	100	100	100	100	100
Hatching day 3 pf (%)	26.7 ± 9.4	28.3 ± 6.4	28.3 ± 14.8	33.3 ± 14.4	26.7 ± 21.1	16.7 ± 3.8

Hatching day 4 pf (%)	90.0 ± 8.6	83.3 ± 15.9	86.7 ± 5.4	76.7 ± 8.6	70.0 ± 12.8	78.3 ± 17.5
Hatching day 5 pf (%)	98.3 ± 3.3	93.3 ± 5.4	98.3 ± 3.3	95.0 ± 6.4	91.7 ± 12.6	96.7 ± 6.7
Hatching day 6 pf (%)	100 ± 0.0	100 ± 0.0	95.0 ± 10.0	100 ± 0.0	98.3 ± 3.3	100 ± 0.0
Hatching day 7 pf (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Normal fry at hatch [%]	100	100	100	100	100	100
Larval survival on day 14 pf [%]	95.0 ± 3.3	90.0 ± 6.7	88.3 ± 6.4	85.0 ± 6.4 *	75.0 ± 11.3 *	70.0 ± 3.8 *
Larval survival on day 21 pf [%]	91.7 ± 6.4	85.0 ± 6.4	83.3 ± 8.6 *	83.3 ± 6.7 *	75.0 ± 11.3 *	68.3 ± 6.4 *
Larval survival on day 28 pf [%]	90.0 ± 8.6	85.0 ± 6.4	83.3 ± 8.6	81.7 ± 8.4	75.0 ± 11.3 *	68.3 ± 6.4 *
Larval survival on day 35 pf [%]	90.0 ± 8.6	85.0 ± 6.4	83.3 ± 8.6	81.7 ± 8.4	73.3 ± 12.4 *	66.7 ± 5.4 *
Larval length (day 35 pf) [cm]	1.40 ± 0.04	1.54 ± 0.05	1.58 ± 0.06	1.57 ± 0.09	1.55 ± 0.09	1.52 ± 0.09
Larval dry weight (day 35 pf) [mg] [1]	5.0 ± 0.3	8.5 ± 1.4	10.1 ± 0.7	9.8 ± 2.5	9.6 ± 2.4	9.2 ± 1.6

[1] *mean single dry weights*

** Statistically reduced compared to the pooled control based on Williams' Test*

No statistically significant treatment related effect could be observed on the fish larvae concerning the endpoint hatching success. For post hatch survival a statistically significant decrease was observed at mean measured concentrations of 939 and 1870 µg a.s./L at test end. Lengths and single dry weights were found to be elevated at all investigated test concentrations. However, the authors proposed that this should not be considered as negative effect on the test organisms. Thus, the lowest no effect concentration, based on the mean measured concentrations, was proposed to be 509 µg a.s./L for the endpoint post hatch survival after 35 days. The EC_{10} and EC_{20} for post hatch survival were calculated to be 390 (95%CL 147 – 645) and 1279 (95%CL 769 – 3877) µg a.s./L, respectively.

RMS comments:

The study fulfils the validity criteria of OECD TG 210 (2013):

• dissolved oxygen concentration should be >60% of the air saturation value throughout the test;

• water temperature should not differ by more than ± 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (Annex 2);

• analytical measurements of the test concentrations.

• overall survival of fertilised eggs and post-hatch success in the controls >70%

In the table below, the RMS has summarised the measured concentrations of Thiophanate-methyl and Carbendazim in the test solutions at different time points during the study. It was noted that in most cases the concentrations of Carbendazim increased during the test, indicating that using the mean measured may be an over-estimation of the initial concentration potentially resulting in effects from short term exposure.

Table 163: Measured concentrations of Thiophanate-methyl and Carbendazim (MBC) in the ELS study under flow-through conditions. Mean of four replicates.

	Thiophanate-methyl Measured (µg/L)						**MBC Measured (µg/L)**					
Treatment group	Con-trol	1	2	3	4	5	Con-trol	1	2	3	4	5
Start	0	113.3	232.5	474.5	926.0	1892.0	0	14.0	19.0	25.3	38.5	59.7
Day 7	0	128.8	264.0	550.0	996.3	1960.3	0	13.4	19.0	25.9	50.0	86.1
Day 13	0	128.0	276.8	537.3	974.8	1960.3	0	13.2	19.4	26.5	47.0	80.0
Day 21	0	121.5	256.3	495.3	906.3	1835.0	0	16.1	23.0	29.1	48.2	78.5
Day 27	0	119.8	240.8	478.8	880.3	1706.8	0	14.9	22.9	31.0	40.6	56.2
Day 35	0	124.0	267.5	519.5	953.8	1863.5	0	16.1	22.2	30.6	40.7	58.9
Mean measured	0	122.5	256.3	509.2	939.5	1869.6	0.0	14.6	20.9	28.0	44.2	69.9
Std dev	0	5.8	16.8	31.3	43.5	94.5	0.00	1.3	2.0	2.5	4.8	13.0

From the results, although not statistically significant at all concentrations, there seems to be treatment related effect on larval survival throughout the test at all treatment levels. Therefore, the RMS would support the EC_{10} (390 µg a.s./L for Thiophanate-methyl, not reported for Carbendazim but may be estimated to be ca 20 µg/L) as endpoint for this effect. The steepness of the curve cannot be calculated in accordance with EFSA 2015 as no EC_{50} could be calculated. However, as the EC_{10} is lower than the lower limit of the EC_{20} the applicant proposed that there is a high certainty of the protection level. The RMS agree with this conclusion regarding the effects on larval survival. Regarding the observed effects on larval length (>10% increase at all treatment levels) and dry weight (>70% increase at all treatment levels), the RMS proposed that this may be a relevant effect. However, this was questioned by EFSA during the commenting period ("*relevance of selecting positive effects on fish for setting a NOEC seems not fully justified*") and it was concluded that the risk assessment should be based on the EC_{10} of 390 µg a.s./L for Thiophanate-methyl. No further discussion was foreseen.

Due to concerns during the review process a statement was provided by the applicant on the impact of the effects seen on fish length and weight in the test item treatment groups of the fish early life stage test with Thiophanate-methyl (Report number 826-002). The conclusion of this statement (Report number 881-021) is submitted below.

Reference:	**xxxx (2017):** Scientific statement on the correlation of fish size and reproduction, for interpretation of observed effects of the following study: Zebrafish (Danio rerio), Early Life Stage Toxicity Test, Flow through conditions
Report number:	881-021
Guideline:	Not relevant
GLP:	Not relevant
Previous evaluation:	No, submitted for the purpose of renewal.

Summary:

In the fish early life stage study (Report number 826-002), increased growth in terms of length and single fish dry weight at test end was observed. The mean length of fish was 14 mm in the control and up to 15.8 mm at a treatment level of 250 µg a.s./L. At the highest treatment level of 2000 µg a.s./L, a mean size of 15.2 mm was observed.

The mean single dry weight in controls was determined to be 5.0 mg. The maximum mean single dry weight of 10.1 mg was observed at a treatment level of 250 µg a.s./L. At the highest treatment level, a mean single dry weight of 9.2 mg was observed.

It was observed that the increased growth in terms of length and weight was not concentration-dependent, as it was observed across treatment levels in a similar range. If the test item would have been responsible for increased size, a dose dependent increase of growth could be assumed in correspondence to increasing Thiophanate-methyl concentrations. Thus, it is assumed that this effect was not treatment related.

An increased size is not considered by the author as a negative effect. A review article by Lawrence (2007) indicates that for example good water quality promotes zebrafish growth and reproduction. Sub-optimal conditions result in a decrease in growth rates and the number and quality of offspring. No correlation of large size and reduced reproductive performance was described. Furthermore, the Lawrence (2007) repeatedly correlated increased growth with an optimal health status of fish. Thus, for unknown reasons, the health status under treatment conditions in Report number 826-002 was rather advantageous compared to control conditions. No hint to any adverse effect related to increased growth rate is given in this review.

Control fish as well as fish under Thiophanate-methyl treatment conditions reached an overall mean length of 14 mm in the GLP-study of Report number 826-002, which exceeded the minimum mean size of 11 mm as suggested by the OECD TG 210. It can thus be postulated that the growth rate observed in this study indicates that test conditions were optimal for fish growth.

There author referred to several studies examining the correlation of reproductive success and size of male and female fish. In many fish species, a relationship exists between female body size and fecundity, following a linear regression (Wootton, 1998). For example, Uusi-Heikkilä *et al*. (2010) reported that large female zebrafish spawned more frequently and had significantly greater clutch sizes than small females. Furthermore, eggs from small fish suffered from higher egg mortality than eggs from large individuals. In another study, it was reported that females showed a clear preference for high quality, i.e. large males (Uusi-Heikkilä *et al*., 2012). In consequence, a higher spawning probability and a larger clutch size were observed. These findings indicate that increased size of fish did not only enhance own survival but also reproductive performance and survival of the offspring. A negative impact of increased size on reproduction could not be concluded from this study.

A detailed comparison of the lengths and weights observed in the fish early life stage study to historical control data was also performed. Only data with comparable number of introduced eggs per vessel (15 to 20 eggs per vessel) were included in order to rule out any potential effect due to different fish density.

Table 164: Historical control data * on fish size

Year of conduct		2013	2012	2014	2016
No of introduced eggs at test start		15	15	20	20
		Control fish Study #1 (NIS-002-4-43-A)	Control fish study #2	Control fish study #3	Control fish study #4
Single dry weight in controls during fish early life stage studies [mg/fish]	A	5.0	4.8	7.9	6.3
	B	5.1	4.6	7.8	5.8
	C	4.6	7.4	8.3	8.3
	D	5.4	7.4	8.6	6.3
	Mean	5.0	6.1	8.2	6.7
	SD	0.3	1.6	0.4	1.1
Single wet weight in controls during fish early life stage studies [mg/fish]	A	24.71	25.00	33.26	27.05
	B	23.66	26.50	33.17	25.18
	C	23.18	33.20	34.23	34.76
	D	23.76	31.90	33.05	26.57
	Mean	23.8	29.2	33.4	28.4
	SD	0.6	4.0	0.5	4.3
Mean length in controls during fish early life stage studies [mm]	A	13.5	13.3	15.5	14.1
	B	14.3	13.2	15.4	13.4
	C	14.3	15.4	15.4	15.4
	D	13.7	14.7	15.0	13.9
	Mean	14.0	14.2	15.3	14.2
	SD	0.4	1.1	0.2	0.9

* Historical control data were derived from FELS studies conducted at the Fraunhofer IME and reared under similar conditions as the study NIS-002/4-43/A

Comparison of the size data of the study of Report number 826-002 to the historical control size data indicate that fish in controls were considerably small compared to controls of similar studies. For example, the single dry weight of controls in the here discussed study are between 4.6 mg and 5.4 mg, while historical control values range between 4.6 mg and 8.6 mg. Fish length of controls of the here discussed study varies between 13.5 mm and 14.3 mm, while historical control values range between 13.2 mm and 15.5 mm. Even though for the test item treatment groups there are still values for length and weight above the values observed in controls of similar studies, the differences are not as prominent as compared to controls of the study of Report number 826-002. The values of the study Report number 826-002, which are above the values of all individual replicates of historical control data, are furthermore distributed across all treatment levels, with highest values at 250 and 2000 µg a.s./L, ruling out any concentration-related effect. Nevertheless, it was proposed that there is no concern about the performance of the study as fish in controls reached the minimum value of 11 mm.

RMS comments:

The statement by the applicant supports the conclusion that the observed positive effect on fish size should not be regarded as biologically relevant. Hence, the EC_{10} of 390 µg a.s./L based on effects on post hatch survival is regarded as the relevant endpoint from this study.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON THIOPHANATE-METHYL (ISO); DIMETHYL (1,2-PHENYLENEDICARBAMOTHIOYL)BISCARBAMATE; DIMETHYL 4,4′-(O-PHENYLENE)BIS(3-THIOALLOPHANATE)

Author:	xxxx
Title:	The effect of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to Salmo gairdneri (Rainbow trout) in a 21-day Prolonged Toxicity Test (method OECD)
Date:	17 Apr 1989
Doc ID:	A40788, Report no: CE89/021; BVL No. 1758283
Guidelines:	OECD 204 (Draft)
GLP:	Yes
Validity:	Yes

Aim was to assess the effects of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Oncorhynchus mykiss* (Rainbow trout, formerly *Salmo gairdneri*) in a 21-day prolonged toxicity test (method OECD).

Methods

Carbendazim; substance, technical; Code: Hoe 017411 00 ZD99 0010, purity 99.4 %. Physical form of test substance: Solid, white crystalline. Lacking stability data are not considered to affect the validity of the study as chemical analysis in test medium showed expected values. Dosing vehicle: 1 NHCL

Test method: OECD 204 (1984)

Test system: Rainbow trout (*O. mykiss*, formerly *Salmo gairdneri*)

Number of animals: 10 fish per tested concentration and control

Age/size/weight: Approx. 10 months /6.1 cm (n = 70)/4.2 g (n = 70)

Maintenance conditions: Stainless steel tanks; photoperiod 16 h light;14° C ±1° C; loading 0.09 g/L - 0.12 cm/L at start and 1.19 g/L - 1.63 cm/L as a mean value.

Test concentrations (flow through):

0 (control and solvent control), 0.0032, 0.0056, 0.010, 0.018 and 0.032 mg/L

Application of test substance: Automatic dosage pumps during flow through phase

Duration of exposure/recording: 21 days

Measured/observed parameters: Chemical analysis in medium: 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21 days after initiation; mortality and symptoms: every 24 hours

Results

Analyses of test substance concentration in medium showed mean recoveries of 94.3 % to 102.7 % during the exposure phase. Hence, all calculations are based on nominal values. The findings of the study are summarized in the table below.

Table 165: Mortality and intoxication symptoms in rainbow trout when exposed to carbendazim for a period of 21 days

Test day	Para-meter	Control	Solvent Control	Carbendazim (nom mg/L)				
				0.032	0.018	0.010	0.0056	0.0032
0	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
1	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
2	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
3	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
4	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
5	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
6	%M IS	0 -	0 *	0 -	0 -	0 -	0 -	0 -
7	%M IS	0 -	0 *	0 S1/10	0 -	0 -	0 -	0 -
8	%M IS	0 -	0 *	0 S1/10	0 -	0 -	0 -	0 -
9	%M IS	0 -	0 *	0 S1/10	0 -	0 -	0 -	0 -
10	%M IS	0 -	0 *	0 S1/10	0 -	0 -	0 -	0 -
11	%M IS	0 -	10 -	0 S1/10	0 -	0 -	0 -	0 -
12	%M IS	0 -	10 -	0 S1/10	0 -	0 -	0 -	0 -
13	%M IS# IS# IS#	0	10	0 S1/10 S3/10 S4/10	0 S4/10	0 S4/8	0 S4/8	0
14	%M IS# IS# IS#	0	10	0 S1/9 S3/9 S4/9	0 S4/10	0 S4/10	0 S4/10	0
15	%M IS# IS# IS#	0	10	0 S1/9 S3/9 S4/9	0 S4/10	0 S4/10	0 S4/10	0
16	%M IS#	0	10	10	0	0	0	0

	IS# IS#			S1/9 S3/9 S4/9	S4/10	S4/10	S4/5	
17	%M IS# IS# IS#	0	10	10 S1/9 S3/9 S4/9	0 S4/10	0 S4/10	0 S4/10	0
18	%M IS# IS# IS#	0	10	10 S1/9 S3/9 S4/9	0 S4/10	0 S4/10	0 S4/10	0
19	%M IS# IS# IS#	0	10	10 S1/9 S3/9 S4/9	0 S4/10	0 S4/10	0 S4/10	0
20	%M IS# IS# IS# IS#	0	10	10 S1/9 S3/9 S5/1 S4/9 S6/9	0 S4/10	0 S4/10	0 S4/10	0
21	%M IS# IS# IS#	0	10	30 S1/7 S3/7 S4/7	0 S4/10	0 S4/10	0 S4/10	0

* One fish appeared to be damaged and died on day 10 in the solvent control group

% M: percent mortality

IS: Intoxication symptoms, type and number of affected fish
S1 delayed uptake of feed and feed residues on the bottom of the test tanks
S2 swimming on back on the surface and slow reaction
S3 indifferent, reduced escape behaviour
S4 fish stay mostly near outflow
S5 narcotic conditions
S6 damaged tail fin and reduced mobility

Table 166: Toxicity endpoints assessed in a fish juvenile growth test with O. mykiss exposed to carbendazim for 21 day

Endpoint	Value (mg/L)	Time (d)	ai	n/r/i
NOEC	0.0032	21		n
LOEC growth	0.032	21		r

n = nominal; r = real; i = initial

The NOEC after 21 days was 0.0032 mg carbendazim/L. Mortality was only observed at the highest concentration level. Differences in size and weight of the fish in comparison to control animals were ascertained to be significantly influenced by the test substance at 0.032 mg/L, only.

Conclusion

Report results listed and evaluated in the carbendazim monograph of 13.11.1997.

The NOEC in a 21 d prolonged toxicity test with rainbow trout *O. mykiss* was 0.0032 mg carbendazim/L. Mortality of 30 % was only observed at 0.032 mg/L, the highest tested concentration. At this concentration, fish size and weight was also influenced in comparison to control animals.

11.6.2 Chronic toxicity to aquatic invertebrates

Reference:	**Handley, J.W. et al. (1990):** An assessment of the effects of Thiophanate-methyl on the reproduction of *Daphnia magna*
Report number:	235/4. RD-9010 (827-001)
Guideline:	OECD TG 202, Part 2 (1984)
GLP:	Yes.
Previous evaluation:	In DAR 1997.
Material and methods:	
Test material:	Thiophanate-methyl
Lot/Batch No:	TP-569 (impurities: 3.0% sodium chloride, 0.69% sulphur
Purity:	95.2%
Species:	*Daphnia magna* (Straus)
Treatments:	0.18, 0.56, 1.8, 5.6 and 18 mg/L (nominal).
Controls:	Untreated and solvent control (DMF, dimethylformamide)
Replication:	Four test vessels per treatment level, 10 daphnids per vessel
Duration:	21 days
Test conditions:	Semi-static, renewal 3 times per week.
	Temperature: 20 - 21°C
	Dissolved oxygen: 7.9 – 8.8 mg/L
	pH: 7.7 – 7.8
	Photoperiod: 16 h light : 8 h dark
	Light intensity: not reported
	Food was supplied continuously.
Observations:	Live and dead animals of the parental generation was counted daily together with the general condition and size compared to the control. The number of daphnids with eggs or young in the brood pouch was determined at each test media renewal together with the numbers of

	live and dead F1 daphnia. The number or discarded unhatched eggs was also determined at this time.
Chemical analysis:	Water samples of fresh media were taken on Day 0 and expired media were taken on Days 2, 4, 7, 9, 11, 14, 16, 18 and 21 from each test and solvent control vessel. Analysis by HPLC.
Data analysis:	One way Analysis of Variance (F_{max} test for homogeneity of variance)

Results:

Measured test concentrations were in the range 51 – 174% of the nominal values. Arithmetric mean measured concentrations were 0.168, 0.486, 1.65, 5.24 and 16.3 mg/L (86.8 – 93.5% of nominal). The results were based on nominal test concentrations.

The biological results are summarised in the table below.

Table 167: Summary of effects on survival and reproductive parameters of Thiophanate-methyl to Daphnia magna.

Nominal test concentration (mg/L)	% survival of P1	% adults with eggs in the brood pouch (%)	No. of live young		No. of dead young		No. of unhatched eggs	
			Total	Per female	Total	Per female	Total	Per female
Control	100	100	3011	75	3	<1	1	<1
Solvent control	100	100	2998	75	0	0	3	<1
0.18	100	100	2916	73	1	<1	14	<1
0.56	100	100	73	2	0	0	1723	43
1.8	98	100	0	0	0	0	1673	43
5.6	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0

There were no significant effects on reproduction of *Daphnia magna* at the test concentration of 0.18 mg/L after 21 days exposure as determined by one-way analysis of variance comparing the numbers of young produced per adult, from the solvent control and test groups.

Apart from the initial acute effect on the parental generation (48 hours EC_{50} for adult survival was reported to be 12.7 mg/L) no other adverse effects of exposure were observed during the study in

those groups which survived through to maturation. All control groups and the 0.18 mg/L produced significant numbers of young by Day 7 and all surviving Daphnia appeared similar in terms of size and condition.

A toxicological significant effect on reproduction was observed in the 0.56 and 1.8 mg/L test groups. Despite the adult generation surviving through to maturation and all the surviving adults in each group showing the presence of eggs in the broad pouch these eggs once shed failed to hatch resulting in significant numbers of unhatched eggs being recorded in these test groups. The numbers of adults showing the presence of eggs in the broad pouch in the 0.56 and 1.8 mg/L test groups was equal to that exhibited by the control groups.

Numbers of unhatched eggs and dead young were low in the controls and 0.18 mg/L test groups.

A NOEC was determined to 0.18 mg/L (nominal) based on the effects on reproduction at 0.56 mg/L and higher test concentrations.

Based on a re-evaluation of the endpoints from this study provided by the applicant during the evaluation (Wirzinger and Ruhnke, 2016, Doc. No. 882-009), the EC_{10}, EC_{20} and EC_{50} values for immobility were 1.63 (95%CL 1.36 – 1.89), 1.96 (95%CL 1.67 – 2.24) and 2.77 (95%CL 2.43 – 3.17) mg/L, respectively. For reproduction, the EC_{10}, EC_{20} and EC_{50} values were 0.190 (95%CL 0.181 – 0.199), 0.215 (95%CL 0.205 – 0.225) and 0.273 (95%CL 0.261 – 0.285) mg/L, respectively. All values were based on geomean measured test concentrations.

RMS comments:

The study was re-evaluated against the validity criteria in OECD TG 211 (2012):

- control mortality of parent animals (female *Daphnia*) does not exceed 20% at the end of the test;

- mean number of living offspring per parent animal surviving in the control at the end of the test is > 60.

These validity criteria were fulfilled, and the study is considered as still valid for risk assessment.

Since individual measured concentrations deviated more than 20% from the control (51 – 174%, see table below), the RMS proposes that the results are based on geomean measured concentrations, resulting in a NOEC of 0.16 mg/L.

Further, it was noted that at Day 0 and Day 21 of the test, levels of test compound was observed in the negative control, 0.036 and 0.043 mg/L, respectively. Given the low margin to the highest concentration with no observed effects, 0.16 mg/L, this may affect the reliability of the results. However, since the statistical analysis was made against the solvent control the results are considered as sufficiently robust.

Table 168: Results of the analytical determination of Thiophanate-methyl concentrations in the control and in the lowest test level.

Nominal test concentration of Thiophanate-methyl [mg/L]	**Day**	**Measured concentration [mg/L]**	**%of nominal values**
Control	0	0.036	-
	2	<0.01	-
	4	<0.01	-
	7	<0.01	-
	9	<0.01	-
	11	<0.01	-
	14	<0.01	
	16	<0.01	-
	18	<0.01	-
	21	0.043	-
0.18	0 (fresh)	0.189 (geomean 0.160)	105
	2 (old)	0.138	77
	4 (old)	0.148	82
	7 (old)	0.144	80
	9 (old)	0.183	102
	11 (old)	0.092	51
	14 (old)	0.156	87
	16 (old)	0.184	102
	18 (old)	0.314	174
	21 (old)	0.135	75

Reference:	**Schäfers, C. (2007):** *Daphnia magna* - Reproduction test (OECD 211) with aging test item - TOPSIN-M WDG
	Amendment: Schäfers 2016, Doc. No. 827-007
Report number:	RD-01133, NIS-003/4-21 (827-006)
Guideline:	OECD TG 211 (1998)
GLP:	Yes

Previous evaluation:	Submitted for the purpose of renewal.
Material and methods:	
Test Material:	TOPSIN M WDG containing 70.5 % a.s.
Lot/Batch #:	G000008868
Test species:	*Daphnia magna* STRAUS, 4 – 24 hours old.
Treatments:	Control, 24, 43, 78, 140 and 252 µg formulation/L
Control:	Dilution water
Replication:	For each test concentration ten replicates with 1 daphnid each were exposed.
Duration:	21 days exposure; started at the peak of formed Carbendazim in the ageing solution.
Test conditions:	Semi-static, with a renewal of the test solutions three times weekly. Test vessels were 60 mL glass beakers, covered with a glass pane. The daphnids were fed during the test with suspensions of unicellular green algae. Temperature: 20.5°C ± 1°C Dissolved oxygen: 8.1 – 8.3 mg/L pH: 7.71 – 8.65 Photoperiod: 16 h light : 8 h dark (600 – 700 lux)
Observations:	The following endpoints observed in the reproduction test were evaluated quantitatively: •Mortality (immobility) of parental generation daphnids •Time to the first brood •Total number of offspring per replicate •Cumulative number of live offspring per surviving female at the time of recording •Intrinsic rate of increase r
Chemical analysis:	Once weekly, samples were taken in old and fresh test medium from each vessel and pooled for replicates 1 to 5 and 6 to 10. The water samples were analysed for Thiophanate-methyl and the metabolite Carbendazim.
Data analysis:	For each endpoint, the NOEC, LOEC were determined. A LOEC was calculated by using ANOVA followed by Williams' test or Mann-Whitney U-test with Bonferroni correction as appropriate non-parametric test as suggested by the ToxRat program. As the test results for reproduction and intrinsic rate showed a concentration-response relationship, the data were analysed by regression to determine the EC_{50} including the 95 % confidence interval as well as the EC_{10} using Probit-analysis assuming log-normal distribution of the values by using a computer program (ToxRat).

Results:

Analytical results

The measured concentrations of Thiophanate-methyl and Carbendazim in the test vessels are presented in the table below. Thiophanate-methyl concentrations increased during the pre-treatment phase depending on the nominal concentration in the aging aquaria. During pre-exposure and exposure, the development of the

formation rate of Carbendazim was similar in all aging aquaria, reaching equilibrium between 60 and 70 % of initial Thiophanate-methyl loadings around day 8, when the *Daphnia magna* reproduction test was started.

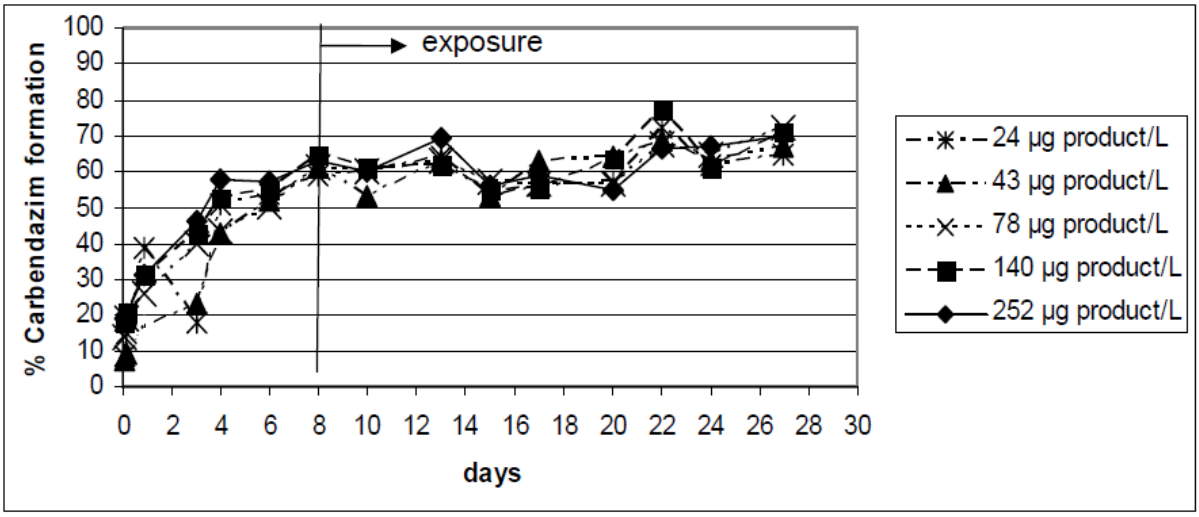


Figure 1: Formation rate of Carbendazim from Thiophanate-methyl (molar equivalents)

Table 169: Analytical results for the determination of the concentrations of Carbendazim during the 21 d exposure period

Nominal Topsin concentration [µg/L]	Nominal Thiophanate-methyl concentration [µg/L]	Measured Thiophanate-methyl concentration at day 0 [µg/L]		Measured Carbendazim concentration during exposure [µg/L]
		Fresh	Old	
24	16.9	23.7	24.7	5.3±0.5
43	30.3	40.4	48.0	9.7±0.9
78	55.0	101.4	101.3	17.7±1.5
140	98.7	178.4	178.4	32.4±3.7
252	177.7	318.3	318.0	57.7±9.2

Fresh: taken from aging tanks before introduction into exposure vessels

Old: taken from exposure vessels immediately before exchange of medium

When re-calculating the applied concentrations of the formulation by adding the active substance equivalents generated by the Thiophanate-methyl and Carbendazim measurements and considering the active substance content of the product, concentrations of the formulation of the freshly prepared and the aged test solutions were 94 – 130 % and 103 – 130 % of nominal concentrations, respectively. The effect concentrations for Thiophanate-methyl were related to nominal test concentrations, which was proposed to be a conservative approach since measured concentrations were higher than the nominal in the day 0 samples. Effect concentrations for Carbendazim were based on mean measured concentrations over the exposure period of 21 days (day 8 – 28). For the latter, arithmetic means were used instead of geometric means, as there was variability due to dynamic equilibrium events or variability of sampling, rather than to dissipation.

All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within the ranges required by the guideline.

Biological results

All validity criteria for the performance of the controls were reported as fulfilled: Survival was above 80 %, the mean number of offspring per female over 21 days was above the criterion of 60. The dissolved oxygen concentration was always above 3 mg/L.

No treatment related mortality occurred up to and including the highest test concentration. Adult body length was not affected. Age at the first brood was significantly increased at the highest treatment. No offspring mortality occurred. The mean cumulative number of offspring showed a treatment-related reduction.

Effects on survival and reproduction are integrated by the intrinsic rate of population increase. The intrinsic rate of population increase was significantly reduced at the two highest test concentrations. The inhibition of reproduction measured as the reduction in the cumulative number of offspring per surviving female was the most sensitive endpoint.

A summary of the observed effects is presented in the table below.

Table 170: Summary of effects on *Daphnia magna* exposed to TOPSIN M 70% WDG for 21 days

Nominal Topsin conc. [µg/L]	Mean conc. Thiophanate-methyl conc. [µg/L]	Mean conc. Carben-dazim [µg/L]	Sur-vival (%)	Growth (length day 21, mm)	Mean number of young per female	Age at first brood [days]	Intrinsic rate of population increase [day^{-1}]	% effect mean number of young /female
Control	-	-	90	4.80±0.25	82.4±19.0	9.6±0.6	0.31±0.05	-
24.0	6.4±1.3	5.3±0.5	100	4.81±0.44	88.7±10.4	9.5±0.0	0.32±0.02	-7.6
43.0	9.8±1.5	9.7±0.9	100	4.91±0.40	76.4±15.6	9.7±0.6	0.31±0.03	7.3
78.0	37.3±3.5	17.7±1.5	100	4.80±0.43	73.6±14.6	10.4±1.4	0.30±0.03	10.7
140.0	63.2±6.0	32.4±3.7	90	4.62±0.30	55.8±14.1*	10.8±1.7	0.27±0.05*	32.3*
252	113.0±7.5	57.7±5.3	100	4.79±0.27	38.7±17.8*	14.3±1.1*	0.22±0.04*	53.1*

* statistical difference to control based on multiple testing

The overall NOEC for the most sensitive endpoint (mean number of young per female) after 21 days was determined to be 78 µg Topsin/L, corresponding to 54 µg Thiophanate-methyl/L (based on nominal, 37.3 µg/L based on mean measured concentrations). The corresponding NOEC for Carbendazim was 17.7 µg/L based on mean measured concentrations, and EC_{10} for the same endpoint was calculated to 14.9 (95% CL 9.9 – 18.9) µg/L.

RMS comments:

The validity criteria of the updated OECD TG 211 (2012) were fulfilled (parent mortality < 20% at the end of the test; mean number of living offspring produced per parent animal > 60. It should be noted that the test compound was a different formulation type than the representative formulation for this evaluation. Information on the composition of both formulations is included in Annex C and a comparison based on the aquatic toxicity of the two formulations was provided by the applicant (Sewell and Mullee, 2002; 822-005, cited by Nieslony and Heidemann, 2007; 881-009). It was concluded that the toxicity of active substance and the formulations is comparable and that the toxicity towards aquatic organisms results only from the active substance itself and not from the formulants within a certain formulation. The formulations revealed comparable acute toxicity, the WDG formulation being slightly higher in toxicity than the SC formulation. Consequently, it was proposed that the higher tier study with *Daphnia magna* which was performed with a Thiophanate-methyl containing WDG-formulation is also representative for the 500 SC formulation. This was tentatively accepted by the RMS, however, it should be noted that the analysis was based solely on comparison of acute toxicity and there may be an uncertainty in extrapolation to chronic toxicity. Member states are asked to give their views on this issue during the peer review.

Based on other available data on aquatic invertebrates, the toxicity of Thiophanate-methyl can be concluded to be lower than that of Carbendazim. The RMS would therefore propose that the effects observed in this test are likely due to the presence of Carbendazim.

The proposed NOEC values from this study were based on statistical significance, however, due to the observed non-statistically significant effects (10.7% reduction in number or young per female) at this treatment level it would be more appropriate to use the EC_{10} as an alternative endpoint for the risk assessment. The EC_{10} for the most sensitive endpoint (mean cumulative number of offspring) was 14.9 (95% CL 9.9 – 18.9) µg/L based on mean measured concentrations of Carbendazim during the exposure phase.

In order to support the use of the chronic *Daphnia magna* study (Schäfers, 2007, see above) conducted with TOPSIN-M WDG (70 % Thiophanate-methyl) for the risk assessment for Topsin M 500 SC (50 % Thiophanate-methyl), the acute *Daphnia magna* study with TOPSIN M 70 % WDG (70 % Thiophanate-methyl), cited by Nieslony & Heidemann (2007), was submitted by the applicant. A summary is given below.

Reference:	**Sewell, I.G., Mullee, D. M. (2002):** TOPSIN M 70% WDG: Acute Toxicity to *Daphnia magna*
Report number:	235/402 (822-005)
Guideline:	OECD TG 202 (1984)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal.
Material and methods:	
Test Material:	TOPSIN M WDG containing 71.8 % a.s.
Lot/Batch #:	540/0000004/13
Test species:	*Daphnia magna*, in-house culture <24 hours old.
Treatments:	Daphnids were exposed to an aqueous dispersion of TOPSIN M 70% WDG at nominal concentrations of 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L
Control:	Freshwater
Replication:	Duplicate vessels, each containing 10 first instars
Duration:	48 hours
Test conditions:	Semi-static. Temperature: 21°C Dissolved oxygen: 8.3 – 8.6 mg/L pH: 7.5 – 7.8 Photoperiod: 16 h light : 8 h dark
Observations:	The number of immobilized Daphnia was recorded after 24 and 48 hours.
Chemical analysis:	Measured concentrations of Thiophanate-methyl were determined at 0, 24 and 48 hours by chemical analysis.
Data analysis:	Probit analysis

Results:

Analytical results

The mean measured concentration of Thiophanate-methyl in the test vessels ranged from 83 to 106 % after test start and was between 60 and 92 % of nominal values after 24 hours. Analysis of the 24 h old preparations at 24 and 48 h showed measured concentrations in a range of 61 to 79 % and 53 to 100 % of

nominal values, respectively. The variation in measured test concentrations was considered to be due to the problems associated with the sampling and analysis of dispersions rather than true solutions. Therefore, the test results were based on the nominal exposure concentrations.

Table 171: Measured Thiophanate-methyl concentrations in the exposure solutions during the 48-hour exposure of *Daphnia magna* to Topsin M 70 % WDG

Nominal concentration [mg product/L]	**Measured concentration [1] [mg product/L]**			
Thiophanate-methyl 70 % WDG	0 hours	24 h (old)	24 h (new)	48 h (old)
0 (control)	< LOQ	< LOQ	< LOQ	< LOQ
0.10	0.088 (88)	0.0605 (61)	0.0602 (60)	0.0525 (53)
0.18	0.190 (106)	0.126 (70)	0.129 (72)	0.117 (65)
0.32	0.270 (84)	0.206 (64)	0.235 (73)	0.178 (56)
0.56	0.482 (86)	0.443 (79)	0.495 (79)	0.386 (69)
1.0	0.831 (83)	0.652 (65)	0.711 (88)	0.588 (59)
1.8	1.58 (88)	1.35 (75)	1.34 (75) / 1.66 (92) *	1.24 (69)
3.2	2.97 (93)	2.46 (77)	2.66 (83)	2.16 (67)
5.6	4.98 (89)	4.27 (76)	3.91 (70) / 4.06 (72) *	3.84 (69)
10	10.2 (102)	7.90 (79)	7.37 (74) / 8.33 (83) *	10.0 (100)

1 values in parentheses represent % of nominal concentration

* analysis of frozen sample

Water quality parameters like temperature, dissolved oxygen and pH value were within ranges requested by guideline OECD 202.

Biological results

After 24 hours, there were no immobile daphnids in the control and in the test item treatment groups up to and including 3.2 mg product/L. After 48 hours, there were no immobile daphnids in the control and in the test item treatment groups up to and including 1.8 mg product/L.

Table 172: Observed mortality of *Daphnia magna* exposed to a WDG formulation containing 70 % Thiophanate-methyl for 48 hours in a static acute test

Nominal Concentration [mg product/L]	**Number of *Daphnia magna***	**Number immobilised (% immobilised)**	
		24 hr	48 hr
Control	20	0 (0)	0 (0)
0.10	20	0 (0)	0 (0)
0.18	20	0 (0)	0 (0)
0.32	20	0 (0)	0 (0)
0.56	20	0 (0)	0 (0)
1.0	20	0 (0)	0 (0)
1.8	20	0 (0)	0 (0)
3.2	20	0 (0)	3 (15)
5.6	20	4 (20)	17 (85)
10	20	10 (50)	20 (100)

The 48-hour no observed effect concentration (NOEC) of TOPSIN M 70% WG to first instar *Daphnia magna* was found to be 1.8 mg product/L; the 48-hour EC_{50} was 4.2 mg product/L (95 % confidence intervals: 3.7 – 4.9 mg product/L), corresponding to 3.02 mg a.s./L (based on nominal concentrations).

RMS comments

The following validity criteria were met: not more than 10 % immobile daphnids in the control (actual 0 %); and the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels (actual ≥ 8.3 mg/L). However, analytical measurements must be considered to be of low reliability due to problems associated with sampling of the dispersed test material. Although not providing any useful endpoint for the risk assessment, the study can be considered as supportive information for the study by Schäfers (2007). The results indicate that the tested WDG-formulation is also representative for the 500 SC formulation, however, it should be noted that the analysis was based solely on comparison of acute toxicity and there may be an uncertainty in extrapolation to chronic toxicity.

Reference:	**Memmert. U. (2002):** Effects of ^{14}C-Thiophanate-methyl on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system
Report number:	831148. RD-II 02133 (828-001)
Guideline:	OECD Draft TG 219 (2001)
GLP:	Yes
Previous evaluation:	Addendum 7 to the Monograph (DAR), 2003.
Material and methods:	
Test material:	Technical Thiophanate-methyl and phenyl-C^{14}-labelled thiophanate-methyl
Lot/Batch No:	NSTM-98824 and EC-01-01
Purity:	99.93% and 96.9%, specific radioactivity 1.64 MBq/mg
Species:	*Chironomus riparius*, first larval stage, 3 days after hatching.
Treatments:	0.125, 0.25, 0.50, 1.00, 2.00 mg/L (nominal), spiked water.
Controls:	Reconstituted water, initial pH ca. 7.9
Replication:	20 larvae per test vessel, 3 replicates.
Duration:	28 days, 7 days after emergence of the last test animal in the control.
Test conditions:	Artificial sediment; 4% sphagnum peat, 20% kaolin clay, 76% sand, 0.26% $CaCO_3$, total organic carbon 2% dry weight. Glass beakers, 600 mL were used as test vessels. 130 g wet sediment (corresponding to 89 g dry sediment) and 250 mL test water was used per beaker. Water temperature 20.0 – 21.8°C, 16 hours light per day (light intensity 560 – 680 Lux). pH was in the range 6.0 – 8.0, and oxygen concentration 6.4 – 8.3 mg/L (>75% of saturation) throughout the test. Fish food was added three times per week.

Observations: Number of emerged adults and their sex was recorded daily from day 10 after application until test termination day 28. Number of visible pupae that had failed to emerge was counted separately for each beaker. Any other signs of intoxication was recorded.

Chemical analysis: Water, pore water and sediment was sampled in duplicate for analysis on day 0, 7 and 28 in the test. Radioactivity was measured by means of LSC, HPLC and TLC.

Data analysis: The emergence ratio and the development time and rate were calculated for each test vessel as the sum of fully emerged midges divided by the number of inserted larvae.

Results:

Measured test concentrations in the water phase were 87 – 89% of the nominal values after 1 hour, 6 – 17% after 7 days, and 0 – 2% after 28 days. The results were based on nominal test concentrations.

Table 173: Mean measured concentrations at different time points during the test.

Nominal (µg a.s./L)	Fraction	Day 0 measured (% of applied)	Day 7 measured (% of applied)	Day 28 measured (% of applied)
500	Parent	A: 87.2 B: n.a. C: n.a.	A: 6.2 B: n.a. C: 2.3	A: not detected B: n.a. C: 4.8
2000		A: 88.7 B: n.a. C: n.a.	A: 17.2 B: n.a. C: 4.6	A: 1.5 B: n.a. C: 4.5
500	CF-27	A: not detected B: n.a. C: n.a.	A: 44.7 B: n.a. C: 35.7	A: 6.0 B: n.a. C: 27.6
2000		A: not detected B: n.a. C: n.a.	A: 24.8 B: n.a. C: 20.8	A: 7.4 B: n.a. C: 21.3
500	2-AB	A: not detected B: n.a. C: n.a.	A: not detected B: n.a. C: not detected	A: 7.9 B: n.a. C: 1.3

2000		A: not detected B: n.a. C: n.a.	A: 7.5 B: n.a. C: 2.8	A: 5.4 B: n.a. C: 5.7
500	M2 unknown	A: not detected B: n.a. C: n.a.	A: not detected B: n.a. C: not detected	A: not detected B: n.a. C: not detected
2000		A: not detected B: n.a. C: n.a.	A: not detected B: n.a. C: 0.6	A: not detected B: n.a. C: 0.9
500	M4 impurity	A: 1.8 B: n.a. C: n.a.	A: not detected B: n.a. C: not detected	A: not detected B: n.a. C: 1.3
2000		A: 1.4 B: n.a. C: n.a.	A: 1.2 B: n.a. C: 2.2	A: not detected B: n.a. C: 1.6
500	Non-extractables	1.4	10.7	46.9
2000		1.9	7.4	39.5

A: water phase

B: sediment pore water

C: sediment extract

Table 174: Summary of results on emergence and development of *Chironomus riparius*, mean of four replicates.

Nominal test concentrations (ug/L)	**Control**	**125**	**250**	**500**	**1000**	**2000**
Initial measured (ug/L)				**436**		**1774**
Number of emerged midges during the test period						
Sum of males	7.5	9.8	10	9.3	12.5	0
Sum of females	11.3	9.3	9.0	10.3	3.4	0
Sum of both sexes	18.8	19.0	19.0	19.5	16.3	0
Emergence ratio						

Nominal test concentrations (ug/L)	**Control**	**125**	**250**	**500**	**1000**	**2000**
Initial measured (ug/L)				**436**		**1774**
Number of emerged midges during the test period						
Sum of inserted larvae per treatment	80	80	80	80	80	80
Sum of emerged midges per treatment	75	76	76	78	65	0
% of emerged midges per treatment (mean)	94	95	95	98	81	0
% of control	100	104	104	107	84*	0*
Development rate per treatment (d^{-1})						
Males	0.066 (0.0017)	0.064 (0.0035)	0.063 (0.0019)	0.065 (0.0039)	0.063 (0.0003)	n.a.
Females	0.062 (0.0027)	0.058 (0.0024)	0.059 (0.0017)	0.061 (0.0006)	0.057 (0.0085)	n.a.

**statistically significant based on William's test (a=0.05, one-sided smaller)*

Based on the results presented above and the nominal concentrations, a 28 d NOEC of 1.0 mg a.s./L was obtained for effects on development and the NOEC for effects on emergence was 0.5 mg a.s./L. The EC_{15} values for emergence ratio and development rate were determined to be 0.952 and > 1.0 mg a.s./L, respectively.

The EC_{10} and EC_{20} values were not reported, but the EC_{15} values were determined. However, the NOEC is the relevant endpoint for the chronic risk assessment for chironomids.

RMS comments:

The study fulfilled the validity criteria of OECD TG 219 (2004):

- emergence in the controls > 70% at the end of the test

- emergence to adults from control vessels between 12 and 23 days after their insertion into the vessels

- oxygen concentration > 60% of the air saturation value, and the pH of overlying water in the range 6-9; the water temperature should not differ by more than ± 1.0 °C.

The results were based on the nominal concentrations in the water phase, although the levels declined to below 20% within a week. Although this is consistent with OECD TG 219, it is

acknowledged that the toxicity might be under-estimated, especially since the major exposure route for *Chironomus* is considered to be via the water phase. The RMS proposes that the measured initial concentration in the water phase is used, resulting in a NOEC of 0.44 mg a.s./L.

Author:	**Kelly, C., Thirkettle, K., Smith, B., Graham, F.H.**
Title:	Carbendazim technical prolonged toxicity to *Daphnia magna*
Date:	15 July 1997
Doc ID:	SNG 80/970692; BVL No. 1840691
Guidelines:	OECD Guideline 202
GLP:	Yes
Validity:	Yes

Semi static chronic toxicity test with *Daphnia magna* (Straus)

Methods

Carbendazim technical, purity 99,5 %

Exposure mode: semi static, solutions were renewed three times per week

Nominal test concentrations:

0, 0.0018, 0.0056, 0.018, 0.056, 0.18 mg/L

Results

Measured test concentrations in the test were:

0, 0.0015, 0.0046, 0.015, 0.045, 0.19 (81 – 106 % recovery)

The carbendazim concentration-response curve regarding the effects on the reproductive performance of *Daphnia magna* shows in the lower concentration range tested (< 0.015 mg/L) a gentle slope (control = 71 juvenile/adult; 0.0015 = -13 %; 0.0046 = -21 %; 0.015 = -23 %). When exposed to the next tested concentration (0.045 mg carbendazim/L), *Daphnia magna* reproduction was reduced by 94 % of control values (5 juveniles/adult). At 0.19 mg/L the mortality was 100% and hence no reproduction could be assessed.

Table 175: Chronic toxicity endpoints for *Daphnia magna* exposed to carbendazim

Endpoint	Value (mg/L)	95 % confidence interval (mg/L)	Time (d)	ai	n/r/i
NOEC reproduction	0.0015		21		r
EC_{50} reproduction	0.025	0.019 – 0.032	21		r
EC_{50} Immobilisation	0.074	0.048 – 0.011	21		r

The acute toxicity of carbendazim was additionally assessed at 48 h after start of the test. The EC_{50} (48 h) for *Daphnia magna* was 0.092 mg carbendazim/L (0.045 - 0.19 mg/L), pointing at a very acute toxic effect of

carbendazim: the difference to the 21 d values are minimal and at 0.019 mg/L the observed 100% mortality could be observed already 2 days after beginning of exposure.

Conclusion

Report results listed and evaluated in the Addendum 8 to the monograph (03 May 2004). The NOEC concerning the reproduction performance of *Daphnia magna* exposed to carbendazim in a chronic test was 0.0015 mg/L. EC_{50} (21 d) reproduction = 0.0025 mg carbendazim/L (0.019 – 0.032 mg/L) and EC_{50} (21 d) immobilisation = 0.074 mg/L (0.048 – 0.011 mg/L).

Author:	Sowig, P., Gosch, H.
Title:	Chronic toxicity to the sediment dwelling chironomid larvae *Chironomus riparius* - Carbendazim; water miscible suspension
Date:	25 Apr 2002
Doc ID:	C018793; BVL No. 1758317
Guidelines:	OECD Draft Guideline No 219 (Draft February 2001)
GLP:	Yes
Validity:	Yes

Methods

Test substance: Formulation AE F017411 00 SC42 A208, Carbendazim 41.6 %

Guideline: OECD Draft Guideline No 219 (Draft February 2001)

Test species: *Chironomus riparius*

Exposure mode: Spiked water

Concentration levels, nominal:

0, 0.001, 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032 mg/L

Results

Measured concentration levels in the water phase: recovery between 69.4 - 74.8 % related to nominal concentrations. Since dissipation of the test item from the water phase to the sediment is to be expected in water-sediment systems, the biological results are based on initial nominal figures.

Table 176: Chronic toxicity of carbendazim to the sediment dweller *Chironomus riparius*

Endpoint	Value (mg/L)	time	ai	n/r/i
NOEC emergence	0.032	28 d		n
LOEC emergence	≥ 0.032	28 d		n

n = nominal; r = real; i = initial

Conclusion

The NOEC for *Chironomus riparius* exposed to carbendazim in a 28 d chronic toxicity test was 0.032 mg /L, corresponding to 0.0133 mg Carbendazim/L.

11.6.3 Chronic toxicity to algae or other aquatic plants

See section 11.5.3 above.

11.6.4 Chronic toxicity to other aquatic organisms

No further data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

According to the CLP criteria a substance should be assigned to hazard class Aquatic Acute 1 (H400) if EC_{50} of the most sensitive organism is lower than 1 mg/L. According to the conclusions above (section 11.5) this is considered fulfilled for thiophanate-methyl including its major environmental metabolite carbendazim. This metabolite was formed as 82.8% after 14 days in study on biodegradation in surface water and as maximum 81.6% after 8 days in study on biodegradation in water/sediment systems. In both test systems (water-only and water/sediment) carbendazim could be observed as >10% already after 1 day. It was also found as maximum 75.6% (day 7) in studies on biodegradation in soil. Carbendazim was also a major product formed in abiotic studies (58.7% after 4 days in hydrolysis study at alkaline pH 9, 22°C, and 49.7% after 5.5 days of exposure to natural sunlight in Japan, mid-December).

The lowest acute toxicity value for the parent compound thiophanate-methyl was 4.4 mg/L, determined with *Daphnia magna.* However, the lowest acute toxicity value for the major metabolite carbendazim was 0.019 mg/L, determined with the fish species *Ictalurus punctatus.* As the EC_{50} is between 0.01 and 0.1 mg/L, a M-factor of 10 according to Regulation EC 1272/2008 is proposed.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Based on available data relevant for classification (section 11.1) it can be concluded that thiophanate-methyl and its major metabolite carbendazim are not rapidly degraded in the aquatic environment. In a ready biodegradability test in accordance with OECD 301C, 4% was biodegraded in 28 days. In biodegradation studies in surface water, in water/sediment systems and in soil, thiophanate-methyl was transformed with half-lives from less than one day up to a few days, while carbendazim was more persistent with half-lives of 64.8 days (water only), 15.1-91.6 days (water/sediment) and 22.0-63.2 days (soil). Carbendazim was found as 82.8% after 14 days in study on biodegradation in surface water, as maximum 81.6% after 8 days in study on biodegradation in water/sediment systems, and as maximum 75.6% after 7 days in studies in soil. According to the CLP criteria a substance should be assigned to hazard class Aquatic Chronic 1 (H410) if NOEC or EC_x of the most sensitive organism is lower than 0.1 mg/L. According to the conclusions above this is considered fulfilled for thiophanate-methyl including its major environmental metabolite carbendazim.

Based on the available data, the substance has a low potential for bioaccumulation.

The lowest chronic toxicity value for the parent compound thiophanate-methyl was the NOEC = 0.16 mg/L, determined with *Daphnia magna.* However, the lowest chronic toxicity value for the major metabolite carb

endazim was the NOEC = 0.0015 mg/L, determined with *Daphnia magna*. As the NOEC is between 0.001 and 0.01 mg/L and thiophanate-methyl does not fulfil the criteria for rapid degradation, a M-factor of 10 according to Regulation EC 1272/2008 is proposed.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The suggested classification for thiophanate-methyl is Acute Category 1 (H400), M-factor 10 and Chronic Category 1 (H410), M-factor 10.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Thiophanate-methyl has an existing harmonised classification of Aquatic Acute 1 and Aquatic Chronic 1.

Degradation

Hydrolysis

Two hydrolysis studies, one photolysis study, one ready biodegradability study (OECD 301C), one surface water simulation study (OECD 309), two water-sediment studies (SETAC Guideline) and three soil studies were available in the CLH Dossier.

In the first study (combined reference from DAR: Soeda and Nomura (1986); Kiesel and Geibel (2015c); Kiesel and Geibel (2015d)), thiophanate-methyl was tested at a concentration of 10 mg/L. In the original report, half-lives at 25 °C were estimated to be 867 days (pH 5), 36 days (pH 7) and 0.7 days (pH 9). Hydrolysis products were identified as carbendazim (MBC), AV-1951 and 2-AB. At 22 °C, MBC was found as maximum 58.7 % (pH 9, last sampling point) and AV-1951 was found as max 26.4 % (pH 9, sampling point at 75 hours). The results of this test were later kinetically reanalysed giving a DT_{50} at pH 7 of 46.8 and of 1 for pH 9. In summary, Thiophanate-methyl is stable to hydrolysis at ambient temperature (22 °C) and pH-values (5–7) but degrades at pH 9 DT_{50} within 1 day.

The second study, (combined reference from DAR: Shiotani (2003b); Kiesel, A., Geibel, E. (2015e); Kiesel, A., Geibel, E. (2015f)), the hydrolysis of carbendazim was assessed at a test concentration of 3 mg/L following OECD TG 111. carbendazim is considered as stable under environmentally relevant conditions (pH, temperature). Degradation at pH 4 and 7 was less than 10 % in all samples after 5 days at 50 °C, but exceeded 10 % in pH 9 samples after 5 days. Therefore, the main test was conducted only at pH 9 where a DT_{50} = 200 days at 25 °C was obtained. The results were kinetically reanalysed giving a DT_{50} = 435 days at 20 °C.

Photolysis

The available data showed that Thiophanate-methyl is photolytically degradable under outdoor conditions (Japan, mid-December), with a half-life of 2.3 days under the

conditions of the test. The quantum yield (ϕ) was determined to be 3.84×10^{-3}. From these results DT_{50}s for water bodies and clear sky conditions at 40 °N latitude were calculated to be 0.99 days (summer), 2.0 days (spring) and 5.0 days (winter). The DS concluded that direct photochemical transformation may contribute to the degradation of Thiophanate-methyl under environmental conditions and that the major product formed is carbendazim, with DX-105 formed in less amounts. Photochemical transformation is not expected to be a significant route of degradation of carbendazim.

Ready biodegradation

In a test carried out following OECD TG 301C, Thiophanate-methyl was found to be not readily biodegradable. The test was done at a concentration of 100 mg/L. Primary degradation was found, probably caused to the largest extent by hydrolysis. The main transformation product was carbendazim, accounting for approximately 18 % of the total recovery after 28 days. Percentage biodegradation was on average 4 % in 28 days.

Degradation in water

In the study on mineralisation in surface water following OECD TG 309 (Hurst, 2015), two concentrations were tested, 10 and 95 µg/L. Results showed almost no mineralisation (≤ 0.5 % AR) and most of the radioactivity remained in the water. Thiophanate-methyl had a relatively short half-life in water (DT_{50} 0.64-2.2 days) and degraded to several known degradants (including MBC, FH-432, 2-AB, CM-0237 and DX-105) and two unknown degradants (UM1 and UM2). The main degradation product was carbendazim, which accounted for more than 59 and 70% of the applied radioactivity by the end of the study. DT_{50}s for this degradant were 64.8 for the low dose and > 1 000 days for the high dose.

Water-sediment degradation studies

The route and rate of degradation of ^{14}C-Thiophanate-methyl was investigated in two equilibrated water/sediment-systems following SETAC guideline (Völkl, 2001). DT_{50}s obtained for Thiophanate-methyl were 3.5 and 1.6 days for the whole system. The main degradation product was carbendazim which was formed as > 80 % of the applied radioactivity and has a DT_{50} = 76.2-91.6 days depending of the water/sediment system. CO_2 formation was very low.

Another study following a SETAC guideline (Koch 2001), assessed the degradation of carbendazim in two water/sediment system, obtaining DT_{50}s of 15.1-75.2. In this test, the formation of $^{14}CO_2$ accounted for 6.0 to 23.0 % AR by the end of the study.

Soil degradation

In Völkl (2002), the route and rate of degradation of Thiophanate-methyl under aerobic conditions was studied in three soils following Draft OECD TG No 307; USA EPA TG 162-1. DT_{50}s calculations were superseded by Kiesel and Geibel (2016a,b). The degradation of Thiophanate-methyl was best described by single first order kinetics in all three soils with DT_{50}s < 1 day (0.48–0.70 d). The substance degraded into carbendazim (max 75.8 %; DT_{50}s = 22-63.2 days) and CM-0237 (DT_{50}s = 2.8–86.5).

Another study (Adam 2014; 2016), which studied the route and rate of degradation of Thiophanate-methyl in one soil reported similar results with a DT_{50} for Thiophanate < 0.29 days and for carbendazim of 43.6 days.

Additional DT_{50} presented in studies with carbendazim as the test substance were in the same range.

Conclusion

In the ready biodegradability test, biodegradation reached only 4 % in 28 days. In degradation studies in water only, in water/sediment systems and in soil, the parent compound is transformed with half-lives from less than one day up to a few days to the major environmental degradant carbendazim. Carbendazim on the other hand, is more persistent with half-lives of 64.8 days, of 15.1-91.6 days and of 22.0-63.2 days in water only, in water/sediment and in soil, respectively. Carbendazim was found as > 80 % in water and water/sediment studies and as > 10 % after only one day. In soil studies, carbendazim was found as max 75.8 %. Additional degradation products were formed but to a lesser extent compared to carbendazim. Based on the available information, Thiophanate-methyl is considered as not rapidly degradable for classification purposes.

Bioaccumulation

Experimental data on bioaccumulation (BCFs) are absent. A log K_{ow} value = 1.45 for Thiophanate-methyl was calculated following OECD TG 107 Shake Flask Method. For carbendazim, a log K_{ow} = 1.5 was reported. Both values are below the CLP cut-off value of 4. Based on this, the potential for bioaccumulation is considered to be low.

Aquatic hazards

Acute Aquatic Toxicity

The available and reliable data on acute toxicity of Thiophanate-methyl, its major environmental degradant carbendazim and additional degradation products to aquatic organisms is summarised in the table below.

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Fish					
Oncorhynchus mykiss	Thiophanate-methyl	Acute 96 hr (flow-through)	Mortality, LC_{50}	11.0 (7.6 – 13) (mm)	Report number 821-001
Ictalurus punctatus	Carbendazim*	96 hr	Mortality, LC_{50}	**0.019** (nom)	Report number A30119; cited in EFSA (2010) *
Oncorhynchus mykiss	Carbendazim*	96 hr	Mortality, LC_{50}	0.19 (nom)	Report number A52914; cited in EFSA (2010) *
Oncorhynchus mykiss	4-OH-TM	96 h, static	Mortality, LC_{50}	>10 (mm, filtrated)	Report number 821-008
Oncorhynchus mykiss	CM-0237	96 h, static	Mortality, LC_{50}	>0.14 (mm, filtrated)	Report number 821-009
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	48 h, flow-through	EC_{50} NOEC	**5.4 (4.4-6.3)** (mm) <4.2 (mm)	Putt, A.E. (1992)
Daphnia magna	BAS 325 10 F	48 h, static	EC_{50} NOEC	4.4 (mm) 3.1 (nom)	Elendt-Schneider (1991)
Daphnia magna	Carbendazim*	48 h	EC_{50}	0.15 (nom)	Fisher (1988), cited in EFSA (2010) *
Daphnia magna	4-OH-TM	48 h, static	EC_{50}	>17.6 (mm, filtrated)	Fujikake, N. (2012)
Daphnia magna	CM-0237	48 h, static	EC_{50}	>0.27 (mm, filtrated)	Fujikake, N. (2012)
Chironomus riparius	4-OH-TM	48 h, static	EC_{50}	>14.6 (mm, filtrated)	Kley, A.. Wydra. V. (2012)
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	E_rC_{50}	37.2 (25.6 – 169) (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	E_rC_{50}	27.3 (22.4 – 33.2) (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	E_rC_{50}	> 11 mg/L (mm)	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	E_rC_{50}	> 15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	E_rC_{50}	> 0.182 (mm)	Baba, K. (2012)
Higher plant					
No data, not needed					

Mm = mean measured

Nom = nominal

The lowest acute endpoint for Thiophanate-methyl corresponds to a test with *Daphnia magna* done according to OECD TG 202 with an EC_{50} = 5.4 mg/L. However, due to the rapid conversion of Thiophanate-methyl to carbendazim (up to ca 80 % in one week in aquatic test systems), the DS proposes that the toxicity of this degradation product is relevant for the classification.

There are two acute fish studies for carbendazim. In the first study, a non GLP test following the ASTM Guideline (Report A30119), the toxicity to fish (*Oncorhynchus mykiss, Ictalurus punctatus* and *Lepomis macrochirus*) of carbendazim at 10, 22 and 22 °C, respectively, was evaluated. Ten animals per tested concentration were used and an EC_{50} (96h) = 0.019 mg/L was obtained for *I. punctatus* (*channel catfish*) showing that this species is more sensitive to the substance than *O. mykiss* (EC_{50} = 0.87 mg/L) and *L. macrochirus* EC_{50} > 3.2.

In the second fish study (report A52914) 10 *Oncorhynchus mykiss* per tested concentration (control, 0.10, 0.14, 0.24, 0.32, 0.42, 0.56, 0.75, 1 and 2.1 mg/L) were exposed to carbendazim in a static system resulting in an EC_{50} = 0.19 mg/L. The results

of this test are considered valid by the DS. The test does not follow any Guideline and is not GLP.

For aquatic invertebrates, Fischer (1988) assessed the toxicity of carbendazim to *Daphnia magna* in a static-acute toxicity test according to OECD TG 202 and following GLP. Four tests (test A, B, C, D) with two different vehicles (HCL and acetone) and with different series of test substance concentrations were performed. Test A had a concentration range from 0.1 to 1 000 mg/L with HCl as a vehicle. In Test B and C, the concentration ranged from 0.001 to 10 mg/L, using acetone as a vehicle. In Test D, tests b was replicated using HCl as vehicle.

Results showed that in the test systems with Acetone as solvent, the NOEC ranged from 0.032 (test system C) to 0.0056 (test system B). However, in these tests the substance recovery ranged between 16 and 3 247. Results of test system A (HCl as solvent) were regarded as invalid, since the control vessel showed a contamination with the tested substance when analysed, even if no immobilization of the daphnids was observed. Test D was considered a valid result and an EC_{50} = 0.15 mg/L was obtained based on nominal concentrations (concentrations within the HCL system remain within ± 20 of nominal).

For algae a test was done following OECD TG 201 and GLP (Bell, 1996). In the test, nominal concentrations of 0.46, 1.0, 2.2, 4.6 and 10 mg carbendazim/L were applied. Measured concentrations were within ± 20 of nominal and validity criteria were fulfilled according to the DS. The toxicity of carbendazim resulted in an EC_{50} (72 h) > 11 mg/L.

Based on the rapid and considerable generation of carbendazim and that this is more toxic than the parent molecule, Thiophanate-methyl, the DS proposes classifying Thiophanate-methyl as Aquatic Acute 1; H400 (M=10) based on the LC_{50} of 0.019 mg/L for carbendazim in *Ictalurus punctatus*.

Chronic Aquatic Toxicity

The available and reliable data on chronic toxicity of Thiophanate-methyl, its major environmental degradant carbendazim and additional degradation products to aquatic organisms, is summarised in the table below.

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Laboratory tests					
Fish					
Danio rerio	Thiophanate-methyl	35 days, ELS, flow-through	NOEC EC_{10}	not reported 0.39 (mm)	Report number 826-002 (2014)
Danio rerio	Carbendazim	35 days, ELS, flow-through	NOEC EC_{10}	Not reported	Report number 826-002 (2014)
Oncorhynchus mykiss	Carbendazim*	21 days, flow-through	NOEC	0.0032 (nom)	Report number A40788, cited in EFSA (2010) *

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.16** (mm) Not reported Not reported	Handley, J.W. et al. (1990) ; Wirzinger and Ruhnke (2016)
Daphnia magna	TOPSIN M WDG (Thiophanate-methyl)	21 d, aged test item*	NOEC EC_{10}	0.0373 (mm) 0.0285 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)
Daphnia magna	TOPSIN M WDG (Carbendazim)	21 d, aged test item*	NOEC EC_{10}	0.0177 (mm) 0.0149 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)
Daphnia magna	Carbendazim*	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.0015** (nom) Not reported Not reported	Kelly et al. (1997), cited in EFSA (2010) *
Sediment-dwelling organisms					
Chironomus riparius	Thiophanate-methyl	28 d, water spiked	NOEC EC_{10} EC_{20}	0.44 (init. meas.) Not reported Not reported	Memmert. U. (2002)
Chironomus riparius	Carbendazim*	28 d, water spiked	NOEC EC_{10} EC_{20}	0.0133 (nom) Not reported Not reported	Sowig & Gosch (2002), cited in EFSA (2010) *
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	NOE_rC	n.d. (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	NOE_rC	3.28 (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	NOEC	Not reported	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	NOEC	15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	NOEC	0.182 (mm, dissolved)	Baba, K. (2012)

In the Bell (1996) study with *P. subcapitata* a $NOEC_{biomass}$ = 2.5 mg/L was reported.

Mm = mean measured

Nom = nominal

As for the acute toxicity, the DS considers carbendazim data relevant to classify the substance.

There are two studies presented for fish chronic toxicity. The first study corresponds to a chronic test with Thiophanate-methyl following OECD TG 210 and GLP (Report 826-002) where the concentrations of carbendazim in the different treatments were also measured. The nominal test concentrations of Thiophanate-methyl were 0.125, 0.250, 0.500, 1.000 and 2.000 mg a.s./L. The mean measured test concentrations were between 93.5 % and 103 % of the nominal values. Thus, the treatment levels were defined as 0.123, 0.256, 0.509, 0.939 and 1.870 mg a.s./L. For carbendazim, the mean concentrations per treatment were found to be between 6.3 and 21.0 % of the carbendazim equivalents of the nominal concentrations of Thiophanate-methyl (the nominal concentrations of carbendazim were calculated using the molar weight quotient of carbendazim: Thiophanate methyl of 0.55838) and were calculated to be 0.0146, 0.0209, 0.0281, 0.0442 and 0.0699 mg carbendazim/L.

The test fulfilled the validity criteria. Results showed an EC_{10} = 0.390 mg a.s./L for Thiophanate-methyl. A NOEC/EC_{10} value for carbendazim was not reported, although the

DS submitter estimated it to be ca. 0.020 mg/L.

In the second study, the effect of carbendazim to 10 months old *Oncorhynchus mykiss* was assessed in a 21-day prolonged OECD TG 204 test resulting in a NOEC = 0.0032 mg/L.

The chronic toxicity of carbendazim to *Daphnia magna* (Schäfers, 2007, 2016) was investigated testing TOPSIN M WDG (70.5 % Thiophanate-methyl) at nominal concentrations of 0.024, 0.043, 0.078, 0.140 and 0.252 mg formulation/L in a test following OECD TG 211. The test duration was 21 days and started at the peak of carbendazim formation in the ageing solution. This occurred around day 8 when carbendazim reached equilibrium between 60 and 70 % of initial Thiophanate-methyl loadings. The test fulfilled validity criteria of the updated OECD TG 211 (2012). The overall NOEC for the most sensitive endpoint (mean number of young per female) after 21 days was determined to be 0.078 mg Topsin/L, corresponding to 0.054 mg Thiophanate-methyl/L (based on nominal, 0.0373 mg/L based on mean measured concentrations). The corresponding NOEC for carbendazim was 0.0177 mg/L based on mean measured concentrations, the EC_{10} for the same endpoint was calculated to 0.0149 (95 % CL 0.0099 – 0.0189) mg/L. In the test, the DS indicated that the toxicity of Thiophanate-methyl is lower than that of carbendazim and proposed that the effects observed in this test are likely due to the presence of carbendazim.

A second study (Kelly *et al*., 1997) measured the toxicity of carbendazim to *Daphnia magna* in a test done according to OECD TG 202. Five concentrations were tested: 0, 0.0018, 0.0056, 0.018, 0.056, 0.18 mg/L. Mean measured concentrations were maintained within ± 20 % nominal (0, 0.0015, 0.0046, 0.015, 0.045, 0.19 mg/L). Results showed a concentration-response curve with a gentle slope regarding the effects on the reproductive performance of *Daphnia magna* in the lower concentration range tested (< 0.015 mg/L). When exposed to the next tested concentration (0.045 mg carbendazim/L), reproduction was reduced by 94 % of control values (5 juveniles/adult). At 0.19 mg/L, the mortality was 100 % and hence no reproduction could be assessed. In this test a NOEC = 0.015 mg/L was obtained.

In addition, a further test studied the Chronic toxicity to *Chironomus riparius* of carbendazim according to OECD TG 219. In the test a formulation with 42.6 % of carbendazim was added at concentrations 0, 0.001, 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032 mg/L. Measured concentration levels were between 69.4-74.8 % to nominal concentrations. Since dissipation of the test item from the water phase to the sediment is to be expected in water-sediment systems, the biological results are based on initial nominal figures. The NOEC for *Chironomus riparius* exposed to carbendazim in a 28 day chronic toxicity test was 0.032 mg /L, corresponding to 0.0133 mg carbendazim/L.

In the case of algae (Bell, 1996), the toxicity of carbendazim resulted in a $NOEC_{biomass}$ = 2.5 mg/L.

According to the DS the lowest acute toxicity value for the parent compound Thiophanate-methyl was 4.4 mg/L, determined with *Daphnia magna*. However, the lowest acute toxicity value for the major degradant carbendazim was 0.019 mg/L for *Ictalurus punctatus*. As the EC_{50} is between 0.01 and 0.1 mg/L, an M-factor of 10 is proposed.

DS conclusion on classification for the environment

Based on available data, the DS submitter concludes that Thiophanate-methyl and its major degradation product carbendazim are not rapidly degraded in the aquatic environment. The substance has also a low potential for bioaccumulation. According to the DS, the lowest chronic toxicity value for the parent compound Thiophanate-methyl was the NOEC = 0.16 mg/L, determined with *Daphnia magna*. However, the lowest chronic toxicity value for the major degradant carbendazim was the NOEC = 0.0015 mg/L, determined with *Daphnia magna*. As the NOEC is between 0.001 and 0.01 mg/L and Thiophanate-methyl does not fulfil the criteria for rapid degradation, an M-factor of 10 is proposed.

Based on the rapid and considerable generation of carbendazim and that this is more toxic than the parent molecule, Thiophanate-methyl, the DS proposes classifying Thiophanate-methyl as Aquatic Chronic 1; H400 (M = 10) based on the $NOEC_{immobility}$ of 0.0015 mg/L for carbendazim in *Daphnia magna*.

Comments received during public consultation

Three Member States (MS) and a Manufacturer commented on the CLH report.

One MS agreed that the substance is not rapidly degradable and the aquatic degradant carbendazim is relevant for hazard classification. However, the MS pinpointed that important data is not available to confirm the validity of various studies of this degradation product. In particular it referred to five studies:

- The acute study with *Ictalurus punctatus*, where it argues that no details are provided for the exposure concentration range, test system media, treatment preparation, control data, validity criteria, etc. This information is relevant to support the reliability of the study and its use as the most sensitive result. It also questioned the relevance of *Ictalurus punctatus* for the test since it is a non-standard species.
    - RAC agrees that the summary provided lacks relevant information which RAC cannot verify. However, the test was performed according to ASTM Guideline and has a reliability of 2. RAC also considers that the species and size used is relevant for acute toxicity. This species is included in USEPA Guidance 850.1075 Fish Acute Toxicity Test, Freshwater and Marine. The study is therefore relevant for classification.
- The acute test with *Oncorhynchus mykiss* (Report A52914), indicating that the study was not conducted according to GLP or a recognised validated test guideline and requesting further information on the reliability of the test, the use of nominal values, recalculation of the endpoint and fulfilment validity criteria.
    - RAC agrees that information to independently assess the reliability, validity of the test, or reasons for recalculation, is missing. The test does not follow any Guideline and is non GLP. The study will be used as supporting information.

- The acute study with *Daphnia magna* (Fischer, 1988), where validity criteria cannot be checked and no justification is provided for the use of nominal values.
    - RAC agrees that the information provided does not allow to proof validity criteria. However, this study is also included in the carbendazim dossier where the test was given reliability 2 and it can be seen that validity criteria are met: 1. In the control, not more than 10 per cent of the Daphnia should have been immobilised or trapped at the surface of the water. 2. The dissolved oxygen concentration at the end of the test should be 60 per cent of the air saturation value at the temperature used. The use of nominal values is also justified since recovery is within ± 20 % of the nominal concentration when HCl was used. This study is considered valid for classification.
- The chronic study for *O. mykiss* using OECD TG 204 which is not a valid Guideline for chronic toxicity assessment. RAC agrees, this test cannot be considered a suitable long-term test. According to ECHA Guidance on Information Requirements and Chemical Safety Assessment tests performed according to OECD TG 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines cannot be considered suitable long-term tests.
- The chronic study for Daphnia (Kelly *et al*., 1997), for which it is unclear if nominal or measured concentrations were used. The MS also asks to check validity against current guidance and for an acute 48-h EC_{50} in support of the Fischer 1988 acute endpoint.
    - Based on the summary provided RAC understands that the endpoint is based on measured concentration. The mortality of the parental generation in the control was 10 % at the end of the test and therefore met the requirement of the guideline of < 20 %. At the same time the number of live neonates produced in controls was 2 829 in total or 71 per adult fulfilling criteria. A 48-h EC_{50} for this test is not provided, however RAC considers the acute test for daphnia valid. In addition, in this test daphnia are fed, a 48h EC_{50} would not be fully representative of acute toxicity. RAC is of the opinion that this test is valid for classification purposes.

Another MS stressed that studies with preparations of the active substance are not relevant for classification. RAC agrees with the comment and is of the opinion that formulation data can be used when the effect of the active substance is clearly determined. In the CLH Report, the following formulations are used for ecotoxicity tests BAS 32510F, Topsin 500 SC, AE F017411 00 SC42 A208 and Topsin M WDG. For the three first formulations the concentration of the a.s (Thiophanate for the two first and carbendazim for the third) is not higher than 50 %. With such a low concentration, RAC considers the endpoint obtained not relevant for classification. In the case of Topsin M WDG, purity of the a.s is > 70 %. However, in the test the effect of the active substance remains unclear since it transforms to carbendazim. RAC also considers the endpoints obtained using formulations are not relevant for classification of the active substance as it is not possible to accurately determine the degree to which the active substance products any observed effects.

The third MS agreed with the proposed classification.

In addition a manufacturer disagreed with the proposed classification as Aquatic Acute 1; H400 with M-factor = 10 based on the lowest endpoint for carbendazim (0.019 mg/L for fish). RAC agrees with the DS in using carbendazim data for classification of Thiophanate-methyl due to its rapid conversion to carbendazim and its higher toxicity.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS and considers Thiophanate-methyl to be not rapidly degradable based on the results of a range of biodegradation studies in various environmental compartments.

Bioaccumulation

With a log K_{ow} = 1.45, below the cut-off value of 4, RAC agrees with the DS and considers the substance non bioaccumulative. For carbendazim a log K_{ow} = 1.5 was reported. Also below the cut-off value of 4.

Acute Aquatic Toxicity

RAC agrees with the DS and considers carbendazim (CAS 10605-21-7) data relevant for classification since Thiophanate-methyl rapidly transforms into this degradation product in water, water-sediment and soil and is more toxic than the parent. Thus Thiophanate-methyl classification will be based on carbendazim data.

In the Thiophanate-methyl dossier there is valid acute data for carbendazim for three trophic levels:

Fish - *Ictalurus punctatus*: LC_{50} (96h) = 0.019 mg/L

Invertebrates - *Daphnia magna*: EC_{50} (48h) = 0.15mg/L

Algae - *Pseudokirchneriella subcapitata* EC_{50} (72h) > 11 mg/L

The lowest endpoint corresponds to *Ictalurus punctatus* LC_{50} = 0.019 mg/L, which is below the cut-off value 1mg/L. In this same study, acute toxicity tests also were conducted with yolk-sac fry, swim-up fry, 0.2 g fry and 1.2 g fingerlings of *O. mykiss* and *I. punctatus*. From these additional endpoints, the most sensitive species was again *I. punctatus* (yolk-sac fry) for which an LC_{50} = 0.007 mg/L nominal was obtained, although RAC concluded that this was not suitable for classification (discussed under In-depth analysis by RAC). Following Table 4.1.0 and Table 4.1.3 of the CLP Regulation, RAC agrees with the DS that Thiophanate-methyl warrants classification as Aquatic Acute 1, M = 10 based on the *Ictalurus punctatus* LC_{50} = 0.019 mg/L (0.01 < $L(E)C_{50}$ ≤ 0.1 mg/L).

For chronic toxicity data is available for two trophic levels:

Invertebrates - *Daphnia magna* NOEC (21d) = 0.0015 mg/L

Algae - *Pseudokirchneriella subcapitata* $NOEC_{biomass}$ (72h) = 2.5 mg/L. RAC acknowledges that growth rate data is preferred to biomass if available.

Chronic toxicity data for fish is not available. RAC considers the test for fish following

OECD TG 204 not suitable for the assessment of chronic toxicity under CLP. In the test with *Danio rerio*, the DS estimated an EC_{10} = 0.02 mg/L for carbendazim. Yet this test was done with Thiophanate-methyl and therefore RAC is of the opinion that this endpoint is not representative of carbendazim toxicity.

Hence, the lowest endpoint corresponds to the test done with *Daphnia magna* NOEC = 0.0015 mg/L, which falls within the 0.001 < NOEC ≤ 0.01 range. As the substance is not rapidly degradable and following Table 4.1.0(b)(i) and Table 4.1.3 of the CLP Regulation, RAC agrees with the DS that Thiophanate-methyl warrants classification as Aquatic Chronic 1, M = 10.

In addition, RAC also assessed the chronic toxicity based on the surrogate approach since there are not adequate chronic data for fish following table 4.1.0(b)(iii) of CLP. Based on the acute most sensitive endpoint for fish, being between 0.01 < $L(E)C_{50}$ ≤ 0.1, classification as Aquatic Chronic 1, M = 10, is warranted.

In summary, Thiophanate methyl in not rapidly degradable, has a low potential for bioaccumulation and **warrants classification as Aquatic Acute 1; H400 (M = 10) and Aquatic Chronic 1; H410 (M = 10).**

Supplemental information - In depth analyses by RAC

Analysis of the Ictalurus punctatus test (Palawski and Knowles, 1986)

The test with *Ictalurus punctatus* (report number A30119) cited in EFSA (2010) refers to a paper done by (Palawski and Knowles, 1986). The paper presents various ecotoxicological studies with carbendazim. The endpoints of two of the tests presented could be relevant for classification.

In an acute "standard" test, Channel catfish (*Ictalurus punctatus*) at 22 °C, Bluegill (*Lepomis macrochirus*) at 22 °C and *O. mykiss* (rainbow trout) at 10 °C, were exposed under static conditions to a logarithmic progression of nominal concentrations of carbendazim for 96 hours in glass jars containing 15L of reconstituted soft water (water hardness 40 to 48 mg/L as $CaCO_3$). In total, ten animals per tested concentration were used. Fish were gradually acclimated to the test conditions over a 3-d period. During the exposure period, the number of dead or affected fish in each test chamber was recorded every 24h. The weight of the fish was 0.3 – 1.2 g. The pH of the test was 7.4. The test was done according to ASTM Guidelines and results showed that *I. punctatus* was the most sensitive species with an LC_{50} (96h) (nominal) = 0.019 mg/L.

In addition, acute toxicity tests also were conducted in standard reconstituted water (pH 7.4; water hardness 40 to 48 mg/L as $CaCO_3$, alkalinity 35 mg/L as $CaCO_3$, temperature 22 °C) with yolk-sac fry, swim-up fry, 0.2-g fry and 1.2-g fingerlings of rainbow trout and channel catfish. The most sensitive endpoint corresponded to an *I. punctatus* yolk-sac fry LC_{50} (96h) (nominal) = 0.007 mg/L. This test could potentially provide data similar to that derived from OECD TG 236 (Fish Embryo Acute Toxicity (FET) Test).

RAC has looked at different aspects to reach a conclusion on the final valid endpoint (see also Palawski and Knowles 1986; ECHA 2016; ECHA and UBA 2017 and Sobanska *et al.* 2018):

- The endpoint LC_{50} = 0.019 mg/L is obtained following a standard test comparable to OECD TG 203.
- However, with the data available it is difficult to assess the embryo test adequacy or make a comparison with OECD TG 236. FET was designed for *Danio rerio* and would need to be adapted for *I. punctatus*.
- The FET has uncertainties related to its predictive capacity and its applicability in the Regulatory Context as a substitute of standard tests. For many chemicals, FET sensitivity is lower than the OECD TG 203 although the reasons why this occurs are unknown. A limited number (Thiophanate-methyl among them) exhibit a higher toxicity in FET with an FET/AFT LC_{50} ratio < 0.1. These may represent substances with a mode of action specific for embryonic development, yet the reasons are unknown. In addition, there are still uncertainties in relation to its applicability domain, etc.
- For the reasons given, it is not recommended to use it as a direct "one-to-one" replacement for the OECD TG 203 and thus to be used alone to meet the information requirements under REACH. It can be used in a weight of evidence approach.

Based on this, RAC is of the opinion that the test resulting in an LC_{50} = 0.019 mg/L is more suitable for classification and labelling.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No available data.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Summary

Comments received during public consultation

Comments

Additional key elements

Key elements

Assessment and comparison with the classification criteria

Comparison with the criteria

Supplemental information - In depth analyses by RAC

Analyses

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

See non-confidential Annex 2 and confidential Annex 3.

In addition to the references included in the annex, study summaries for the references in the below table were derived from the Draft Re-Assessment Report (dated July, 2009) produced by Germany in their re-evaluation of carbendazim (major metabolite to thiophanate-methyl) under Regulation 1107/2009.

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
KIIA 7.1.1, 7.2.1, 7.2.2	Otto, S.	1975	Verhalten des Pflanzenschutzmittelwirkstoffes im Boden k.A. , A22991 GLP: N, published: N	N	Y		CTF
KIIA 7.1.1, 7.2.1, 7.2.2	Gildemeister H.; Jordan H. J.; Remmert U.	1981	Behaviour of the plant protection product Hoe 17411 0F AT 102 (carbendazim) in soil SS 2.2 at 15°C, 20°C and 25°C k.A. , A47457 GLP: N, published: N	N	Y		CTF
KIIA 7.8.3	Knoch E.	2001	Degradability and fate of (U-14C-phenyl) car-bendazim in the aquatic environment (wa-ter/sediment system) Aventis CropScience GmbH, DEU, Oekochem-ie, Frankfurt , C017201 GLP: Y, published: N	N	Y		CTF

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
KIIIA1 9.4	Zillgens B.	2007a	Predicted concentrations in soil for carbendazim in various formulations - A modeling study conducted with Microsoft® Excel and Model MakerTM 4.0 E.I. du Pont de Nemours and Company, DuPont-21656 GLP: N, published: N	N	Y		CTF
KIIIA1 9.7	Zillgens B.	2007c	Predicted environmental concentrations of carbendazim in surface water and sediment associated with uses in arable crops: Modeling for the European Union E.I. du Pont de Nemours and Company , DuPont-23452 GLP: N, published: N	N	Y		CTF
IIA 8.2.1/02, IIA 8.2.4/02, IIA 8.2.6/02	xxxx	1984	Toxicological Studies of Benomyl and Carbendazim in Fish A30119 GLP: not applicable Published: yes 1758267	Y	Y		CTF
IIA 8.2.1/03	xxxx	1976	Acute (96-hour) toxicity of H-9910 to rainbow trout A52914, Report no: HLO486-76 GLP: not applicable Published: no 1840602	Y	N		AVO
IIA 8.2.3/01	xxxx	1989	The effect of carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Salmo gairdneri* (Rainbow trout) in a 21-day Prolonged Toxicity Test (method OECD) A40788, Report no: CE89/021 GLP: yes Published: no 1758283	Y	Y		CTF

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
IIA 8.3.1/03	Fischer, R.	1988	The Effect of Carbendazim - substance, technical (Identification code: Hoe 017411 OF ZD99 0010) to *Daphnia magna* (Waterflea) in a Static-Acute Toxicity A39285; Report no: OEK88/091E GLP: yes Published: no 1758297	N	Y		AVO
IIA 8.3.2/05	Kelly,C., Thirkettle, K., Smith, B., Graham, F.H.	1997	Carbendazim technical prolonged toxicity to *Daphnia magna* SNG 80/970692 GLP: yes Published: no 1840691	N	Y		SIN
IIA 8.4/04	Bell, G.	1996	Carbendazim technical: Algal growth inhibiton SNG 44(a)/960463 GLP: yes Published: no 1843621	N	Y		SIN
IIA 8.5.2/01	Sowig, P., Gosch, H.	2002	Chronic toxicity to the sediment dwelling chironomid larvae *Chironomus riparius* - Carbendazim; water miscible suspension C018793 GLP: yes Published: no 1758317	N	Y		CTF

* CTF = Carbendazim Task Force (DuPont de Nemours (Deutschland), BASF Aktiengesellschaft, Bayer CropScience AG), according to information in Draft Re-Assessment Report (dated July, 2009) produced by Germany.

AVO = Hoechst Schering AgrEvo GmbH

SIN = Sinon EU Corporation

Additional references

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15 ANNEXES

Annex 1 Detailed study summaries on the toxicity data referred to in this CLH report

Annex 2 References: List of studies relied upon

Annex 3 CONFIDENTIAL References: List of vertebrate studies relied upon

Annex 4 CONFIDENTIAL Detailed information on tested formulated products.