Committee for Risk Assessment

RAC

Annex 1

Background document

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

Tolclofos-methyl (ISO); *O*-(2,6-dichloro-p-tolyl) *O,O*-dimethyl thiophosphate

EC Number: 260-515-3
CAS Number: 57018-04-9

CLH-O-0000001412-86-266/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:

Tolclofos-methyl (ISO); O-(2,6-dichloro-p-tolyl) O,O-dimethyl thiophosphate

EC Number: 260-515-3

CAS Number: 57018-04-9

Index Number: 015-113-00-0

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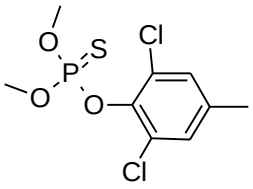
Version number: 2 **Date: June 2018**

CONTENTS

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)	O-2,6-Dichloro-p-tolyl O,O-dimethyl phosphorothioate
Other names (usual name, trade name, abbreviation)	O-(2,6-Dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate
ISO common name (if available and appropriate)	Tolclofos-methyl
EC number (if available and appropriate)	260–515–3
EC name (if available and appropriate)	O-(2,6-dichloro-p-tolyl) O,O-dimethyl thiophosphate
CAS number (if available)	57018–04–9
Other identity code (if available)	479
Molecular formula	$C_9H_{11}Cl_2O_3PS$
Structural formula	
SMILES notation (if available)	Cc1cc(c(c(c1)Cl)OP(=S)(OC)OC)Cl
Molecular weight or molecular weight range	301.1 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant–tolclofos-methyl does not contain any stereoisomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant – Tolclofos-methyl is no UVCB
Degree of purity (%) (if relevant for the entry in Annex VI)	960 g/kg

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)**
Tolclofos-methyl (ISO); O-2,6-Dichloro-p-tolyl O,O-dimethyl phosphorothioate	Min. 96%	Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410	Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410

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
Methanol	0.1%	Flam. Liq. 2 H225 Acute Tox. 2 H301 Acute Tox. 3 H311 Acute Tox 3. H331 STOT SE 1 H370**	-	No

None of the other impurities present in the tolclofos-methyl as manufactured are considered toxicologically or environmentally relevant and are not presented further (i.e. confidential)

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
There are no additives in technical Tolclofos-methyl	-	-	-	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	015-113-00-0	tolclofos-methyl (ISO); *O*-(2,6-dichloro-p-tolyl)-*O*,*O*-dimethyl thiophosphate	260-515-3	57018-04-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS09 GHS07 Wng	H317 H410			
Dossier submitters proposal	015-113-00-0	tolclofos-methyl (ISO); *O*-(2,6-dichloro-*p*-tolyl) *O*,*O*-dimethyl thiophosphate	260-515-3	57018-04-9	**Modify** Skin Sens. 1B **Retain** Aquatic Acute 1 Acute Chronic 1	**Retain** H317 H400 H410	**Retain** GHS09 GHS07 Wng	**Retain** H317 H410		**Add** M = 1 M = 1	
Resulting Annex VI entry if agreed by RAC and COM	015-113-00-0	tolclofos-methyl (ISO); *O*-(2,6-dichloro-*p*-tolyl) *O*,*O*-dimethyl thiophosphate	260-515-3	57018-04-9	Skin Sens. 1B Aquatic Acute 1 Acute Chronic 1	H317 H400 H410	GHS09 GHS07 Wng	H317 H410		M = 1 M = 1	

Table 6: 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 lacking	No
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 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 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	Conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Conclusive but not sufficient for classification	Yes
Carcinogenicity	Conclusive but not sufficient for classification	Yes
Reproductive toxicity	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Conclusive but not sufficient for classification	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Tolclofos-methyl has a harmonised classification in Skin sens. 1, 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 Xi; R43, R50/53 by the ECB as presented in Commission Directive 2001/60/EC.

The substance was discussed at the Meeting of Technical Committee C&L on the Classification and Labelling of Dangerous Substances in Arona (21-24 September), 2004. In the report (ECBI/139/04 Rev. the Technical Committee C&L agreed to classify Tolclofos-methyl with R43 -N; R50-53. This classification was sent to DG ENV with the draft proposal for a 30th ATP.

RAC general comment
Tolclofos-methyl is an active substance in the meaning of Regulation EC 1107/2009. Its use in plant protection products is as a contact fungicide for the control of *Rhizoctonia*. Tolclofos-methyl has an existing entry in Annex VI of the CLP Regulation. This CLH proposal aims at modifying the existing classification based on data submitted as part of the pesticide renewal process (partly old, partly new as compared to the original application).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Tolclofos-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

Tolclofos-methyl is an active substance used in plant protection products. It is used as a contact fungicide for the control of *Rhizoctonia.* The representative uses for the renewal of approval of tolclofos-methyl includes potatoes, lettuce and ornamentals.

6 DATA SOURCES

Tolclofos-methyl was included in the Annex I of the Council Directive 91/414/EEC concering placing of plant protection products on the market in 2007. Toclofos-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 487/2014 of 12 May 2014 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of the active substances Bacillus subtilis (Cohn 1872) Strain QST 713, identical with strain AQ 713,

clodinafop, metrafenone, pirimicarb, rimsulfuron, spinosad, thiamethoxam, tolclofos-methyl and triticonazole

The data presented in this dossier has been submittet by the applicant Sumitomo Chemical Agro Europe S.A.S. 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.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White crystalline solid (100%)	Walker, J. A. (2014), Report No. QP-0131 (Doc. No.: 111-001)	
	White solid at 22°C (97.9%)	Reitz, G. A. (2010), Report No. QP-0118 (Doc. No.: 119-001)	
	Crystalline solid (97.9%)	Asada, Y. (1996), Report No. QP-0072 (Doc. No.: 994-01009)	
	White colour (97.9%)	Asada, Y. (1996), Report No. QP-0073 (Doc. No.: 994-01008)	
Melting/freezing point	Melting point: 78.1°C – 79.3°C. With 99.3% pure	Reitz, G. A. (2010), Report No. QP-0118 (Doc. No.: 119-001)	EC A.1 Capillary tube in a metal block method
Boiling point	Decompose before boiling.	-	
Temperature of decomposition or sublimation	Decomposition at 120°C – 220°C.	Bates, M. (2001), Report No. QP-0094 (Doc. No.: 994-01002)	
Relative density	Density: 1.53×10^3 kg/m^3 at 24°C	Walker, J. A. (2014), Report No. QP-0131, (Doc. No.: 111-001)	
Vapour pressure	1.37×10^{-4} Pa at 10°C (direct) 8.77×10^{-4} Pa at 20°C (direct) 1.82×10^{-3} Pa at 25°C (by interpolation) 3.71×10^{-3} Pa at 30°C (direct)	Hayes, P. C. (2001), Report No. QP-0100 (Doc. No.: 994-01004)	
Surface tension	72.7 mN/m at 20°C 90% saturated solution.	Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)	The study is still acceptable, but however not relevant since the tested substance has a solubility below 1 mg/L i.e. the water solubility for Tolclofos-methyl in distilled water is 0.708 mg/L, see Water Solubility below. (EC A.5 states that "substances with water solubility lower than 1 mg/L

Property	Value	Reference	Comment (e.g. measured or estimated)
			need not be tested")
Water solubility	Water solubility in double distilled water at 20°C is 0.708 mg/L pH ranged between 7.0-7.5	Concha, M. (2001), Report No. QP-0095	
n-Octanol/water partition coefficient **active substance**	pH 7: log Pow 3.8 at 25°C	Bondarenko, S. (2010), Report No. QP-0117 (Doc. No.: 114-002)	OECD 117, HPLC method
Flash point	Not applicable. The active substance is a solid; its melting point is > 40 °C.	-	-
Flammability	Not highly flammable The test substance did not ignite. (Moisture content was 0.04%)	Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)	
Explosive properties	Tolclofos-methyl is considered not to be potentially explosive based on the chemical structure and associated thermodynamic properties.	Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)	Valid waiver
Self-ignition temperature	No self-ignition below 120°C up to the point of decomposition.	Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)	
Oxidising properties	The technical material is not oxidising.	Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)	Test according to EC A.17
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation product	No data		
Dissociation constant	No dissociation constant at environmental relevant pH		
Viscosity	Not relevant since the substance is a solid with a melting point >> 40 °C.		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Expert judgement	Tolclofos-methyl is considered not to be potentially explosive.		Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)

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

Tolclofos-methyl is considered not to be potentially explosive based on the chemical structure and associated thermodynamic properties. A structural comparison was done with the typical phosphores and auxoploses listed in CLP.

8.1.2 Comparison with the CLP criteria

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

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed due to lack of data

8.2 Flammable gases (including chemically unstable gases)

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

8.3 Oxidising gases

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

8.4 Gases under pressure

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

8.5 Flammable liquids

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

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10	Not highly flammable The test substance did not ignite. (Moisture content was 0.04%)		Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)

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

The substance did not ignite in the initial screening test which is the same as in the recommended method in CLP. The test substance should thus not be classified as a flammable solid under CLP.

8.6.2 Comparison with the CLP criteria

The preliminary screening test in EC 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.

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 Tolclofos-methyl does not contain any functional groups known to confer self-reactive properties (compared with Tables A6.1 and A6.2 in Appendix 6 to UN-MTC). It contains the structural feature P-O , but none of the functional groups given in the examples. The waiving criteria 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.

8.8 Pyrophoric liquids

Hazard class not applicable (Tolclofos-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 is available. However, Tolclofos-methyl has been handled in air within all studies available in the dossier and there are no reports of self-ignition.

8.9.2 Comparison with the CLP criteria

Tolclofos-methyl is not a pyrophoric solid

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.

8.10 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A.16	No self-ignition below 120°C up to the point of decomposition.		Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)

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 is available.

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, Tolclofos-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 Tolclofos-methyl it is not a substance which in contact with water emit flammable gases (compare with CLP guidance section 2.12.3).

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

8.12 Oxidising liquids

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

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A.17	The technical material is not oxidising.		Bates, M. (2001), Report No. QP-0099 (Doc. No.: 994-01021)

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

One test performed in accordance with EC A.17 is available. None of the tested ratios of test substance:cellulose mixtures (10-90% test substance) ignited.

8.13.2 Comparison with the CLP criteria

The test was done in accordance with EC A.17 which is not in line with the test scheme in CLP with respect to type of reference used and ratios . However, since the test substance did not ignite in any of the mixtures tested no classification is warranted.

8.13.3 Conclusion on classification and labelling for oxidising solids

Data conclusive but not sufficient for classification.

8.14 Organic peroxides

Hazard class not applicable (Tolclofos-methyl is not an organic peroxide).

8.15 Corrosive to metals

No data has been provided addressing this property.

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

No data has been provided addressing this property. Tolclofos-methyl does not contain acidic or basic functional groups. Additionally, the substance is a high melting 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.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Based on the chemical structure, tolclofos-methyl is not considered potentially explosive or self-reactive. Tolclofos-methyl is not flammable or oxidising, and is not reported (experience in handling) to self-ignite or, upon contact with water, to emit flammable gases. Therefore, the Dossier Submitter (DS) concluded that no classification is required.

Comments received during public consultation

One MSCA commented that the purity of the test substance could be added for each property presented in Table 7 of the CLH report.

Assessment and comparison with the classification criteria

Tolclofos-methyl does not have flammable, pyrophoric or oxidising properties and does not emit flammable gases upon contact with water. RAC therefore supports **no classification for these physico-chemical properties**, as proposed by the DS.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 12: Summary table of toxicokinetic studies

Method, guideline, test substance, species, strain, no./group	Results	Remarks	Reference
In-house method in compliance to OECD 417 but with several deviations [4-methyl-^{14}C]tolclofos-methyl Oral Rat (Sprague Dawley) and Mouse (ICR) Number of animals not indicated	The oral absorption in rat is estimated to be 68 % and in mice 83 %. Tolclofos-methyl is rapidly metabolised and excreted mainly via urine. Tissue residues 7 days after dosing were generally very low (<1% of the high dose). Major urinary metabolites is ph-COOH, ph-CH_3, TMO-COOH and DM-TMO-COOH in both rat and mice and also ph-CO-glycine in mice.	This study is just supplementary Deviation from OECD 417: only one singe dose, no individual animal data, no statitistical analysis, time points when the excretion samples were collected not indicated, the rats were 5 weeks old instead of 6-12 weeks old. Not GLP	RAR Vol. 3 B.6.1.1/01
OECD TG 417 [phenyl-^{14}C]tolclofos-methyl Oral (stomach intubation) Rat (Sprague Dawley) 5/sex/dose, control 3/sex 5 and 200 mg/kg bw single doses and 5 mg/kg bw repeated dose	Tolclofos-methyl was rapidly excreted in rats, mainly in the urine. The oral absorption is 90 % based on excretion in urine. Tissue residues 7 days after dosing were generally very low (<1% of the high dose). The major metabolites found were DM-TMO, DM-TM-CH_2OH, DM-TM-COOH, DM-TM and TMO-COOH.	Acceptable GLP	RAR Vol. 3 B.6.1.1/02
In-house method in accordance with 88/302/EEC [phenyl-^{14}C]tolclofos-methyl Oral (stomach intubation) Rat (Sprague-Dawley) <u>Tissue distribution study:</u> 3/time point/sex, 7 time points <u>Biliary excretion study:</u> 3/sex Single administration 5 mg/kg bw	The absorption from the gastrointestinal tract was rapid and is estimated to be about 50-70% (after 48 hrs) The major metabolites of tolclofos-methyl found in the bile were ph-CH_3-glucuronide and DM-TM-CH_2OH-glucuronide.	This study is just supplementary Derivation from OECD 417: only 3 instead of 4 animals and no exhaled CO_2 was measured. Not GLP	RAR Vol. 3 B.6.1.1/03

Method, guideline, test substance, species, strain, no./group	Results	Remarks	Reference
Comparative *in vitro* metabolism study No existing guideline [phenyl-^{14}C]tolclofos-methyl Incubation of human and rat microsomes 10 µM for 10 min	The two major metabolites in human and rat microsomes were TM-CH_2OH and TMO-CH_2OH	Acceptable GLP	RAR Vol. 3 B.6.1.1/04

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

Absorption: The results of the rats and mice metabolism studies indicate that most of the ^{14}C Tolclofos-methyl-derived radioactivity was rapidly eliminated by male and female rats at both high (200 mg/kg bw) and low (5 mg/kg bw) dose levels, and by male and female mice at the low dose level. Most of the radioactivity was eliminated within first 48 hours. The similarities in elimination rates for all groups and the fact that urinary excretion is the primary route of elimination, indicate that the processes of absorption and elimination are relatively unaffected at dosages up to 200 mg/kg bw. The absorption from the gastro-intestinal tract was supposed to be relatively rapid and is estimated to be 90 % of the administered doses, based on the radioactivity of the urine metabolites found in the rat (B.6.1.1/02) (follows OECD guidance and is GLP).

Metabolite profile: In both rats and mice, Tolclofos-methyl was mainly metabolised via oxidative desulfuration of the P=S group to P=O, oxidation of 4-methyl group, and cleavage of P-O-aryl and P-O-methyl linkages.

The major metabolite in both rats and mice was ph-COOH. This metabolite was excreted as the glycine conjugate in mice and as the free from in rats.

A comparative *in vitro* metabolism study with rat and human liver microsomes confirmed the main metabolic pathways and revealed no relevant species differences in metabolism. For rat and human liver microsomes, the main metabolites (> 85% of total radioactivity) were TM-CH_2OH, TMO-CH_2OH, ph-CH_3 (2,6-dichloro-p-cesol), ph-CH_2OH and TM-COOH. They presented in rats 11, 43, 10, 10, 11% and in humans 35, 17, 8, 16 and 9% of the total radioactivity, respectively.

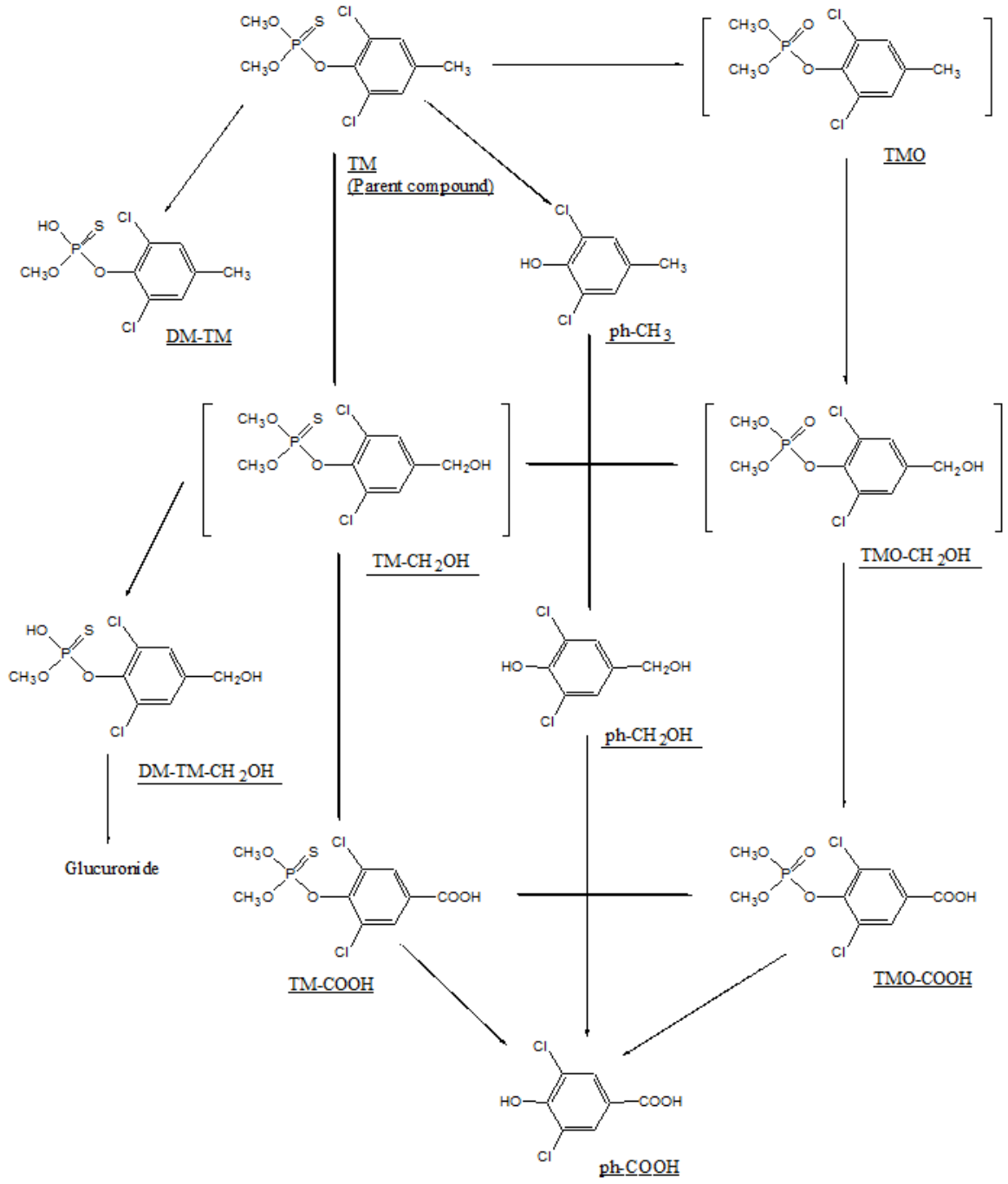
Distribution: Distribution of Tolclofos-methyl into tissues is small and persistence of the compound over a long period is unlikely.

Excretion: Tolclofos-methyl was readily excreted in rats and mice, mainly in the urine. Less than 1% of the dose was retained in the tissues after 7 days. In another study, excretion into bile in the bile-duct cannulated rats showed that cumulative excretion over 48 hours was 5.8 to 11.7% of the dose of ^{14}C in the bile, 46.7 to 59.4% in the urine and 42.3 to 23.7% in the faeces, in males and females respectively (dose level: 5 mg/kg bw). Only the parent compound was detected in faeces and bile when collected 0 to 24 hours after administration. The unabsorbed Tolclofos-methyl was therefore supposed to be excreted without being degraded in the gastro-intestinal tract.

The different studies provided slightly different schemes of proposed metabolic pathways for tolclofos-methyl in rat. The scheme from study B.6.1.1/03 can be seen below. It contains all major metabolites.

Comment: The oral absorption value for tolclofos-methyl was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Experts´consultation 2.1). Oral absorption in mice and rats was considered higher than 90%.

Proposed metabolic pathway for tolclofos-methyl in rat



10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 13: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral In house method in accordance with 92/69/EEC B.1 GLP: No	Rat Sprague Dawley M, F 10/sex/dose	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil	1000, 2500, 3750, 5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw (M, F)	RAR Vol 3 B.6.2.1/01
Acute oral In house method in accordance with 92/69/EEC B.1 GLP: Yes	Rat Sprague Dawley F 10/dose	Tolclofos-methyl (purity: 98.6%) Vehicle: maize oil	200, 5000 mg/kg bw (single dose) 2 mg/kg bw/day (dose given on four occasions two hours apart) formulated at 0.005%, 2% and 50% in maize oil Animals killed 8 hrs after the dosing	>5000 mg/kg bw (fasted F)	RAR Vol 3 B.6.2.1/02
Acute oral FIFRA § 81-1 No GLP but a statement of QA	Rat Sprague Dawley M, F 5/sex/dose	Tolclofos-methyl (purity: 97.7%)	5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw (M, F)	RAR Vol 3 B.6.2.1/03
Acute oral In house method in accordance with 92/69/EEC B.1 GLP: No	Mouse dd M, F 10/sex/dose	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil	1000, 1500, 2000, 3000, 4000 mg/kg bw Observations in 14 days	3500 mg/kg bw (M) 3600 mg/kg bw (F)	RAR Vol 3 B.6.2.1/01
Acute oral (gelatine capsules) In house method in accordance with 92/69/EEC B.1 GLP: No	Dog Beagle M, F 2/sex/dose	Tolclofos-methyl (purity: 98.7%)	100 and 1000 mg/kg bw (animals fasted) 215 and 464 mg/kg bw (animals unfasted) Observations in 16 days	>1000 mg/kg bw (M, F)	RAR Vol 3 B.6.2.1/04

M: male
F: female

Table 14: Summary table of human data on acute oral toxicity

No data

Table 15: 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 (no-GLP, in house method) was conducted in the rat and mouse according to an inhouse method. Groups of ten male and ten female rats (Sprague Dawley) received dosage levels of up to 5000 mg/kg bw of the test substance formulated in corn oil, while groups of ten male and ten females dd mice received dosage levels up to 4000 mg/kg bw of the test substance formulated in corn oil. The animals were observed for 2 weeks. In rats, at the highest dose level of 5000 mg/kg bw, five of ten males and six of ten females were found dead within 1-5 days post-treatment. Toxic symptoms such as decrease of spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body developed 3-4 hours after administration. The minimum toxic dose level was 3750 mg/kg bw for both sexes. At gross necropsy, no visible lesions were observed. The oral LD_{50} values of tolclofos-methyl were about 5000 mg/kg bw in male and female rats. In mice, the toxic symptoms were stated to be similar to those of rats. The minimum toxic dose level in mice was 1500 mg/kg bw for both sexes. No lesions were observed at gross necropsy. The oral LD_{50} values of tolclofos-methyl were 3500 mg/kg bw (males) or 3600 mg/kg bw (female) in mice (RAR Vol. 3, B.6.2.1/01).

In another acute oral toxicity study (no GLP statement but QA), single doses of the test substance suspended in corn oil were administered to groups of Sprague Dawley rats at a dose level of 5000 mg/kg bw. Then the animals were observed for 14 days. No mortalities were observed. Piloerection and abnormal body carriage (hunched posture) were observed in all rats shortly after dosing. Recovery as judged by external appearance and behaviour was apparently complete by day 5. Bodyweight gains were observed in all rats on days 8 and 15. Terminal autopsy findings were normal. The oral LD_{50} for male and female rats was >5000 mg/kg bw (RAR Vol. 3, B.6.2.1/03).

An acute oral toxicity study was conducted according to GLP. The purpose of the study was to assess the systemic toxicity and anti-acetylcholinesterase activity of technical tolclofos-methyl to the female rat following a single oral dose. Groups of ten female rats (Sprague Dawley) received dosage levels of 2, 200 and 5000 mg/kg bw of the test substance formulated at 0.005%, 2% and 50% in maize oil. Ascess to food only was prevented overnight prior to dosing. A volume of 10 ml/kg bw was administered orally by gavage, on one occasion to each rat receiving 200 and 5000 mg/kg bw, and on four occasions (two hours apart) to rats receiving 2 mg/kg bw. All animals were killed eight hours after dosing which was eight hours after the fourth dose for rats receiving 2 mg/kg bw. There were no treatment-related deaths. Piloerection was observed in all rats one hour after dosing and throughout the remainder or the majority of the remainder day. This sign was accompanied three hours after dosing or later by: hunched posture in all surviving rats from all groups, ungroomed appearance and greasy fur in all rats at 2 mg/kg bw, liquid faeces in all rats at 2 mg/kg bw and all reviving rats at 200 mg/kg bw, partially closed eyes in all rats at 5000 mg/kg bw. Clinical signs were still evident at study termination. No body weight losses occurred. Group mean acetylcholinesterase levels (plasma, RBC and brain) were comparable for all three treated groups. No gross lesions were present in the rats at necropsy. The oral LD_{50} for female rats was >5000 mg/kg bw (RAR Vol. 3, B.6.2.1/02).

No deaths were noted in Beagle dogs orally administered tolclofos-methyl as a single dose in gelatine capsules at 100 (animals fasted overnight), 215, 464 or 1000 (animals fasted overnight) mg/kg bw. Clinical signs such as emesis were noted after oral administration at ≥464 mg/kg bw, and stools or diarrhea were observed at ≥100 mg/kg bw. Furthermore, reduced brain cholinesterase activity was noted in dogs at 1000 mg/kg bw (Males: 7% compared to 100 mg/kg bw group; Females: 25% compared to 100 mg/kg bw group). No gross necropsy findings were observed (RAR Vol. 3, B.6.2.1/04)

10.1.2 Comparison with the CLP criteria

According to the CLP Guidance, 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 was above 2000 mg/kg bw and tolclofos-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 tolclofos-methyl.

10.2 Acute toxicity - dermal route

Table 16: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal In house method in accordance with 92/69/EEC B.3 GLP: No	Rat Sprague Dawley M, F 10/sex/dose	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil	1000, 2500, 5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/01
Acute dermal OECD TG 402 GLP: Yes	Rat Sprague Dawley (Crl:CD(SD)) M, F 5/sex/dose	Tolclofos-methyl (purity: 97.5%)	2000 mg/kg bw Observations in 14 days	>2000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/03
Acute dermal In house method in accordance with 92/69/EEC B.3 GLP: No	Mouse dd M, F 10/sex/dose	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil	1000, 2500, 5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/01
Acute dermal FIFRA § 81-1 No GLP but a statement of QA	Rabbit New Zealand White M, F 5/sex/dose	Tolclofos-methyl (purity: 97.7%)	2000 mg/kg bw Observations in 15 days	>2000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/02

M: male
F: female

Table 17: Summary table of human data on acute dermal toxicity

No data

Table 18: 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 in the Sprague-Dawley rat was conducted according to GLP and OECD TG 401. There were no deaths in any animals in the 2000 mg/kg bw dose group. During the observation period, no clinical abnormalities were evident in any animals in the 2000 mg/kg bw group. There were no statistically significant differences in body weight when comparing the treated group to the solvent control group. No abnormalities were observed in necropsy. Based on the results of this study the dermal LD_{50} of tolclofos-methyl was considered to be >2000 mg/kg bw (RAR Vol. 3, B.6.2.2/03).

In another study (no GLP, in house method), tolclofos-methyl was tested for acute dermal toxicity in Sprague Dawley rats and dd mice. The animals were exposed to tolclofos-methyl up to 5000 mg/kg bw (10/sex/dose). The test substance was suspended with corn oil. Surgical tape which was taken away 24 hr after treatment, and the area was cleaned up with absorbent cotton dipped in diethyl ether. All animals were observed for mortality, general condition and clinical signs. Observations days were 14 days. Gross post-mortem examinations were conducted on all animals in the study. At the highest dose level of 5000 mg/kg bw, neither deaths nor remarkable symptoms were seen in rats. In the case of mice, the toxic symptoms such as decrease of spontaneous motor activity, piloerection, loss of appetite, irregular respiration and slight motor ataxia were developed 3 to 4 hrs after administration, at the dose level of 2000 mg/kg bw and above. At higher dose level of 4000 mg/kg bw and above, death of mice was observed within 24 hrs post-treatment and the mortality was 20% and 30% for male (4000 mg/kg bw) and female (5000 mg/kg bw), respectively. The toxic signs of surviving animals disappeared in 3 to 5 days. At gross necropsy, no visible lesions were observed. The dermal LD_{50} values of tolclofos-methyl in rats and mice were determined to be greater than 5000 mg/kg bw (RAR Vol. 3, B.6.2.2/01).

In an acute dermal toxicity study (no GLP, in house method) tolclofos-methyl was administered at 2000 mg/kg bw to skin of New Zealand White rabbits (5/sex/dose). At the end of the 24-hr exposure period, the dressings were carefully removed and the treated area of skin decontaminated by washing in warm water and blotting dry with absorbent paper. Animals were observed soon after dosing, then at frequent intervals for the remainder of Day 1. On subsequent days the animals were observed at least twice. Clinical signs were recorded at each observation. The treated area of skin were examined daily for signs of dermal irritation and assessed for erythema and eschar formation and oedema formation. All animals were observed for 15 days after dosing. One female rabbit was found dead on Day 5. This animal showed no clinical signs on Days 1 to 3. On Day 4 moderate nasal exudate and noisy respiration was observed. Autopsy revealed congestion of the lungs and pallor of the liver and kidneys. A slight bodyweight loss was recorded for this rabbit. In view of the delayed onset of clinical signs and the absence of clinical signs in any of the other treated rabbits at the time it is thought that the death of this rabbit was unlikely to be related to tolclofos-methyl although the exact cause of death was not established. One female rabbit did not eat normally during days 9 to 13 and showed piloerection on day 11. Recovery was completed by day 14. Terminal autopsy findings were within normal limits. The dermal LD_{50} value of tolclofos-methyl in rabbits was found to be greater than 2000 mg/kg bw (RAR Vol. 3, B.6.2.2/02).

10.2.2 Comparison with the CLP criteria

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with dermal LD_{50} of 1000-2000 mg/kg bw. The LD_{50} for dermal toxicity was above 2000 mg/kg bw, and tolclofos-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 tolclofos-methyl.

10.3 Acute toxicity - inhalation route

Table 19: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Acute inhalation In house method in accordance with 92/69/EEC B.2 GLP: Yes *The study was checked for compliance with OECD TG 403. Following deviation was noted: The mass median aerodynamic diameter, MMAD was outside the upper range recommended in the guideline* Study limited since the MMAD was >4 µm	Rat Wistar M, F 5/sex/dose	Tolclofos-methyl (purity: 97.4%) Dust 52% of airborne particles were 5.5 µm or less in aerodynamic diameter	0, 1.35, 3.32 mg/L 4 hr, whole body 14 days observation	>3.32 mg/L (maximum attainable concentration) (M, F)	RAR Vol. 3 B.6.2.3/01
Acute inhalation OPPTS 870.1300 GLP: Yes *The study was checked for compliance with OECD TG 403. Following deviation was noted: dose level used in this study was not the maximum attainable concentration*	Rat Sprague Dawley M, F 5/sex/dose	Tolclofos-methyl (purity: 97.4%) Dust MMAD: 3.6 µm (geometric standard deviation: 2.2 µm)	2.07 mg/L (mean achived atmosphere concentration) 4 hr, nose-only 14 days observation	>2.07 mg/L (M, F)	RAR Vol. 3 B.6.2.3/02

Table 20: Summary table of human data on acute inhalation toxicity

No data

Table 21: 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. Five rats/sex/group were exposed nose-only for a single 4- hour period to atmospheres containing Tolclofos-methyl TG at a concentration 2.07 mg/L (mean achieved atmosphere concentration). The nominal chamber concentration was 6.03 mg/L. Particle size distribution (mass median aerodynamic diameter, MMAD) was calculated to be 3.6 µm and geometric standard deviation 2.2 µm. All animals were observed for mortality during the exposure period. The animals were observed for signs of gross toxicity and behavioural changes upon removal from the exposure tube and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea and coma. Individual body weights were recorded prior to treatment on the day of exposure and on Days 1, 3, 7 and 14 (termination). At the end of the 14-day observation period, the surviving animals were euthanized via carbon dioxide inhalation, and gross necropsy examinations of all animals were performed. Tissues and organs of the thoracic and abdominal cavities were examined. No animals died during the course of the study. Following exposure, all rats exhibited irregular respiration, but recovered from this symptom by day 3. Two of the male animals showed dry rales after removal from the exposure chamber up to a post-exposure period of 2.5 hrs. Although some slight weight losses were observed in most rats due to the exposure procedure the body weights recovered to pre-exposure values or above in 3 males and in 3 of the female animals by day 3 and for all other animals by day 7. The body weight gain for this animals were within the limit for normal expectation. The acute inhalation LC_{50} in male and female rats exceeded 2.07 mg/L. No deaths were observed. An abnormal respiration was observed at the concentration of 2.07 mg/L, but the animals recovered from this symptom by Day 3. No abnormalities were observed in gross necropsy. No gross abnormalities were noted for any of the animals when necropsied (RAR Vol. 3, B.6.2.3/02)

In another study male and female Wistar rats (5/sex/dose) were exposed to tolclofos-methyl atmosphere concentrations of 0, 1.35 and 3.32 mg/L for 4 hours (whole body exposure). The latter concentration was the highest technically achievable concentration. Particle size distribution analysis showed that approximately 52% of the airborne particles were 5.5 µm or less in aerodynamic diameter. The rats were observed continuously for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period. All rats were weighed daily from the day of delivery until the end of the observation period. Food and water consumption was measured daily from five days before exposure. At the end of the 14-day observation period, the rats were anaesthetised and killed by exsanguination. The rats were subjected to a detailed macroscopic examination. The lungs, liver and kidneys were examined microscopically. There were no deaths during the study. Signs consistent with exposure to high concentration of a mildly irritant dust were noted, including closing or partial closing of the eyes, abnormal body position and abnormal breathing. Abnormal respiratory pattern was observed immediately after exposure. No other significant signs were noted during the observation period. Small losses of body weight or a reduction in rate of gain over 3 days following exposure to tolclofos-methyl were observed, with marginal reduction in food consumption. There were no observable abnormalities at gross necropsy. The lung weights to body weight ratios were within normal limits. The microscopic examination showed no treatment-related changes. The LC_{50} (4-hour) for tolclofos-methyl is in excess of 3.32 mg/l of air. The study was considered limited since the mass median aerodymainc diameter was >4 µm (RAR Vol. 3, B.6.2.3/01)

10.3.2 Comparison with the CLP criteria

According to the CLP Guidance, 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 >2.07 mg/L (highest dose tested). The particle size range used in the study (MMAD: between 1 and 4 microns) corresponds to the dose of about 2 mg/l (ideally dose level to be tested in this range in rats). Thus, tolclofos-methyl does not trigger classification for acute inhalation toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification is proposed for tolclofos-methyl.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral

The CLH report included four oral acute toxicity studies.

In a non-GLP study using an in-house (OECD 401-like) method (Anon. 1978, RAR Vol.3 B.6.2.1/01), Sprague Dawley rats (10/sex/group) and dd mice (10/sex/group) received single doses of up to 5000 (rats) or 4000 (mice) mg/kg bw tolclofos (in corn oil) by gavage. The oral LD_{50} values of tolclofos-methyl were about 5000 mg/kg bw in rats and 3500 and 3600 mg/kg bw in male and female mice, respectively. Mortality occurred within 1-5 days after treatment, with 5000 mg/kg bw as the minimum lethal level in rats, and 1500 and 2000 mg/kg bw in male and female mice, respectively. Toxic symptoms such as decreased spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body developed 3-4 hours after administration in rats. In mice, the toxic symptoms were reported to be essentially similar to those in rats.

In another non-GLP study using an OECD 401-like method (Anon. 1985, RAR Vol.3 B.6.2.1/03), no deaths were observed in Sprague Dawley rats (5/sex) treated with 5000 mg/kg bw tolclofos-methyl (in corn oil) by gavage, resulting in an LD_{50} > 5000 mg/kg bw. Piloerection and abnormal body carriage (hunched posture) were observed in all rats shortly after dosing, but the animals had completely recovered by day 5.

In a GLP compliant study which was aimed at determining the acetylcholinesterase activity of tolclofos-methyl (Anon. 1994, RAR Vol.3 B.6.2.1/02), (fasted) female Sprague Dawley rats received a single oral dose of 2, 200 or 5000 mg/kg bw tolclofos-methyl (in maize oil) by gavage. There were no treatment-related deaths (LD_{50} > 5000 mg/kg bw), but it is noted that animals were killed 8 hours after dosing instead of after a 14-day observation period. Clinical signs included piloerection, hunched posture, ungroomed appearance, greasy fur, liquid faeces, and partially closed eyes. Brain, erythrocyte and plasma acetylcholinesterase levels were comparable for all three dosage groups, with erythrocyte and plasma levels showing no consistent changes compared to pre-treatment

levels.

No deaths were observed in an acute oral toxicity study (Anon. 1978, RAR Vol.3 B.6.2.1/04; non-GLP, in-house method) in (fasted) Beagle dogs (2/sex/group) given single doses of 100, 215, 464 or 1000 mg/kg bw tolclofos-methyl (in gelatin capsules), resulting in an LD_{50} > 1000 mg/kg bw. Clinical signs such as emesis and diarrhoea were observed. Furthermore, brain acetylcholinesterase activity was reduced in male and female dogs at 1000 mg/kg bw when compared to the other dose groups (25% and 24%, respectively, compared to males and females at 100 mg/kg bw). Erythrocyte and plasma acetylcholinesterase activity were not affected upon treatment.

As the oral LD_{50} was above the upper boundary for classification of 2000 mg/kg bw, the DS proposed no classification for acute oral toxicity.

Dermal

Three dermal acute toxicity studies were included in the CLH report.

In an OECD 402 and GLP compliant study (Anon. 2010, RAR Vol.3 B.6.2.2/03), no deaths or clinical abnormalities were observed in Sprague Dawley rats (5/sex) treated dermally for 24 hours with 2000 mg/kg bw tolclofos-methyl (moistened with water).

In a non-GLP study using an in-house (OECD 402-like) method (Anon. 1978, RAR Vol.3 B.6.2.2/01), Sprague Dawley rats (10/sex/group) and dd mice (10/sex/group) received dermal doses of 1000, 2500 or 5000 mg/kg bw tolclofos-methyl (suspended with corn oil) for 24 hours. No mortalities or other symptoms were observed in rats, nor in mice (in contrast to the description given in the CLH-report on the findings in mice which does not match the findings described in the RAR).

In the third study (Anon. 1985, RAR Vol.3 B.6.2.2/02; non-GLP, OECD 402-like method), a dose of 2000 mg/kg bw tolclofos-methyl was administered dermally to New Zealand White rabbits (5/sex) for 24 hours. One female died on day 5. As no clinical signs were noted before day 4 and no clinical signs were observed in the other rabbits (aside from piloerection in one female on day 11), no relationship with treatment was assumed for this death. The dermal LD_{50} in rabbits was found to be greater than 2000 mg/kg bw.

The DS proposed no classification for acute dermal toxicity, as the dermal LD_{50} was above 2000 mg/kg bw, which is the upper boundary for classification.

Inhalation

Two acute inhalation studies were presented, both in rats.

In a GLP compliant study using an in-house (OECD 403-like) method (Anon. 1986, RAR Vol.3 B.6.2.3/01), groups of 5 Wistar rats/sex were exposed to tolclofos-methyl atmosphere concentrations of 1.35 or 3.32 mg/L for 4 hours (whole body exposure). No deaths were observed up to and including the highest technically achievable concentration of 3.32 mg/L (with 52% of particles having an aerodynamic diameter ≤ 5.5 µm). Signs consistent with exposure to high concentrations of a mildly irritant dust were noted, including closing or partial closing of the eyes, abnormal body position and abnormal breathing. There were no observable abnormalities at gross necropsy. The LC_{50} was > 3.32 mg/L, but the DS considered the study limited, since the mass median aerodynamic diameter (MMAD) was outside the upper range (4 µm) recommended in OECD 403.

In a more recent GLP compliant study (Anon. 2012, RAR Vol.3 B.6.2.3/02), Sprague Dawley rats (5/sex) were nose-only exposed for 4 hours to 2.07 mg/L tolclofos-methyl (mean achieved atmosphere concentration; MMAD 3.6 µm). The study was consistent with OECD 403, except for the test concentration not being the maximum attainable concentration. No deaths were observed, resulting in an LC_{50} > 2.07 mg/L. Irregular respiration was observed in all animals following exposure, but this had recovered by day 3. Two males also showed dry rales up to 2.5 hours post exposure. No abnormalities were observed at gross necropsy.

As the 4-hour LC_{50} was > 2.07 mg/L and this was the highest concentration tested with the appropriate particle size range, the DS considered that tolclofos-methyl does not trigger classification for acute inhalation toxicity (with a limit of 1-5 mg/L for category 4 for dusts and mists). Thus no classification was proposed.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Oral

RAC agrees with the DS that the oral LD_{50} values found in the acute toxicity studies with rats, mice and dogs do not trigger classification. This is supported by the findings in three oral acute neurotoxicity studies in rats (see section on 'Specific target organ toxicity – single exposure (STOT SE)' below for details), where no mortalities were observed at doses up to and including 2000 mg/kg bw. **Hence, no classification for acute oral toxicity is warranted**.

Dermal

RAC agrees with the DS that **no classification for acute dermal toxicity is warran**ted, given that the LD_{50} values in the three available studies were above the classification limit of 2000 mg/kg bw.

Inhalation

No mortality was observed at the highest concentration tested with an appropriate particle size (2.07 mg/L). Although this concentration is not the maximum attainable and below the upper boundary for classification (5 mg/L for dusts and mists), RAC notes it matches the ideal maximum concentration to be tested in rats (which is 2 mg/L, according to CLP 3.1.2.3.2). RAC therefore considers the 4-hour LC_{50} of > 2.07 mg/L to not warrant classification. This is supported by the fact that also at the highest practically attainable concentration of 3.32 mg/L no mortalities were observed, although the particle size at this concentration was slightly higher than recommended in the test guideline.

There RAC supports no classification for acute inhalation toxicity as proposed by the DS.

10.4 Skin corrosion/irritation

Table 22: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Dermal irritation In house method in accordance with 92/96/EEC B.4 GLP: No	Rabbit Albino rabbits of native Japanese strain 6 males	Tolclofos-methyl (purity: 97.0%)	0.5 g/animal, 4 hours	No erythema, eschar or oedema formation was observed. PI-Score was 0.0	RAR Vol. 3 B.6.2.4/01
Dermal irritation OECD TG 404 GLP: Yes	Rabbit New Zealand White 3 males	Tolclofos-methyl technical (purity: 97.5%) Vehicle: corn oil	0.5 g/animal Examination after 1, 24, 48 and 72 hours	Erythema (score 1) and oedema (score 1) were observed in all 3 animals one hour after removal of the patches. Erythema (score 1) was observed in all 3 animals and oedema (score 1) was observed in 2 animals 24 hours after removal of the patches. After 48 hours erythema was still observed in 2 animals. PI-Score was 1.1 Oedema reactions had disappeared 48 hours and erythema reactions had disappeared 72 hours after removal of the patches.	RAR Vol. 3 B.6.2.4/02
Dermal irritation In house method No GLP but a statement of QA	Rabbit New Zealand White 6 females	Technical Tolclofos-methyl (purity: not specified)	0.5 g/animal Examination after 1, 24, 48 and 72 hours	None of the animals showed any observable response to treatment throughout the observation period. Scores for erythema and oedema: 0.0	RAR Vol. 3 B.6.2.4/03

Table 23: Summary table of human data on skin corrosion/irritation

No data

Table 24: 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 OECD TG 404, 3 male New Zealand White rabbits each received dermal treatments with 0.5 g of tolclofos-methyl moistened with corn oil for 4 hours under occlusive conditions. Approximately 1 hour after removal of the test patches, the test sites were evaluated for

erythema, oedema, and other evidence of dermal effects and were scored according to the Draize scale. Additional evaluations were made at approximately 24, 48 and 72 hours after removal of the patches. Tolclofos-methyl was mildly irritating to the skin of rabbits. Mean values of 0.67 (erythema/eschar and oedema) and 0.33 (oedema) were calculated in 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal. Oedema reactions had disappeared 48 hours and erythema reactions had disappeared 72 hours after removal of the patches. The primary irritation score was 1.1 (RAR Vol. 3, B.6.2.4/02).

Animal number	Score (erythema and eschar/edema)				Mean irritation score (Erythema)	Mean irritation score (Oedema)	P.I.I*
	Time after removal of the patch						
	1 hour	24 hours	48 hours	72 hours			
1	1/1	1/1	1/0	0/0	0.67	0.33	1.1
2	1/1	1/0	0/0	0/0	0.33	0	
3	1/1	1/1	1/0	0/0	0.67	0.33	

In a skin irritation study (no-GLP) six male albino rabbits of native Japanese strain receved dermal treatments with 0.5 g of tolclofos-methyl for 4 hrs under occlusive conditions. Observations were made after 4, 24, 72 hours and 7 days post application. No erythema, eschar or oedema formation was observed. The primary irritation score was 0.0 (RAR Vol. 3, B.6.2.4/01).

No. of animals	Irritant reaction in skin	Site of skin	Intensity of irritant reaction					PI-Score*
6			Observation time					
			4 hrs	24 hrs	48 hrs	72 hrs	7 days	
	Erythema and eschar	Intact	0	0	0	0	0	0.0
		Abraded	0	0	0	0	0	
	Edema	Intact	0	0	0	0	0	
		Abraded	0	0	0	0	0	

In another study (no-GLP), six female New Zealand White rabbits received dermal treatment with 0.5 g of tolclofos-methyl for 4 hrs under semi-occlusive conditions. Observations were made after 30 minutes and 24, 48 and 72 hrs after removal of the test material. Skin reactions of erythema and edema were scored 1, 24, 48 and 72 hours after removal of the patches according to the method of Draize. No erythema or oedema formation was observed. Scores for erythema and oedema were 0.0 (RAR Vol. 3, B.6.2.4/03).

Rabbit No.	E=Erythema O=Oedema	Day			
		1*	2	3	4
1	E	0	0	0	0
	O	0	0	0	0
2	E	0	0	0	0
	O	0	0	0	0
3	E	0	0	0	0
	O	0	0	0	0

4	E	0	0	0	0
	O	0	0	0	0
5	E	0	0	0	0
	O	0	0	0	0
6	E	0	0	0	0
	O	0	0	0	0

10.4.2 Comparison with the CLP criteria

According to the CLP Guidance Table 3.2.2, a substance should be classified in Category 2 (Irritant) *if*:

"-mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

-inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above"

In a skin irritation test conducted with tolclofos-methyl oedema/eschar and oedema was noted. However, the mean value was below 2.3. Furthermore, no inflammation persisted to the end of the observation period. Thus, tolclofos-methyl does not fulfil the classification criteria for skin irritation.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed for trolclofos-methyl.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Three dermal skin irritation studies were available, all in rabbits and involving applying 0.5 g tolclofos-methyl on the skin for 4 hours. In two non-GLP studies using in-house (OECD 404-like) methods, only scores of 0 were observed for erythema and oedema. One study involved six male rabbits of native Japanese strain, with test substance application under occlusion (Anon. 1978, RAR Vol.3 B.6.2.4/01). In the other study, six female New Zealand White rabbits were treated under semi-occlusive conditions (Anon. 1985, RAR Vol.3 B.6.2.4/03). In a GLP and OECD 404 compliant study (Anon. 2010, RAR Vol.3 B.6.2.4/02), tolclofos-methyl (moistened with corn oil and applied under occlusive conditions) caused slight erythema (grade 1) and oedema (grade 1) in all three male New Zealand White rabbits 1 hour after patch removal. The effects were reversible within

24 to 72 hours. Mean individual scores over 24-72 hours were 0.67, 0.33 and 0.67 for erythema and 0.33, 0 and 0.33 for oedema.

The DS concluded that tolclofos-methyl does not fulfil the classification criteria for skin irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Only in one out of three available skin irritation studies in rabbits slight irritation was observed. Given that the mean individual scores for erythema and oedema in this study were both well below the cut-off of 2.3 for classification and the effects were reversible, RAC agrees with the DS that **no classification is warranted for skin corrosion/irritation**.

10.5 Serious eye damage/eye irritation

Table 25: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Eye irritation In house method in accordance with 92/69/EEC, B.5 GLP: No	Rabbit Albino rabbits of native Japanese strain 3-5 males	Tolclofos-methyl (purity: 97.0%)	50 mg/animal, Examination after 1, 24, 48 and 72 hours	No irritant reactions were observed in conjunctiva, cornea and iris of rabbit eyes.	RAR Vol. 3 B.6.2.5/01
Eye irritation In house method in accordance with 92/69/EEC, B.5 GLP: No	Rabbit New Zealand White 6 females	Tolclofos-methyl (purity: 97.7%)	0.1 mL/animal Examination after 1 hr, 1, 2, 3, 4 and 7 days	No corneal damage or iridial inflammation was observed in any of the animals. Transient mild conjunctival reactions with slight to moderate discharge were observed in sex animals. The eyes were normal one or two days after instillation. The mean score for conjunctivae redness is 0.06. The mean score for cornea iris, conjunctivae chemosis and discharge is 0.0.	RAR Vol. 3, B.6.2.5/02
Eye irritation OECD TG 405 GLP: Yes	Rabbit New Zealand White 3 males	Tolclofos-methyl (purity: 97.5%)	0.1 mL/animal Examination after 24, 48, 72 and 96 hrs	No cornea or iris reactions were observed after test material application. For the conjunctiva, redness (score 1) and chemosis (score 1) were observed in all 3 animals one hour after application of the test material. Redness (score 1) in the conjunctiva in all 3 animals and chemosis (score 2) and discharge (score 1) in the conjunctiva in one animal were observed 24 hours after application in one animal. Forty-eight hours after application, redness (score 1), chemosis (score 1) and discharge (score 1) were observed in one animal. Redness (score 1) and chemosis (score 1) were observed in this animal 72 hours after application. These reactions had disappeared 96 hours after application of the test material. Mean irritation scores: Conjunctival redness: 1, 0.33, 0.33 Conjunctivae chemosis: 1.33, 0, 0.33 Cornea and iris: 0	RAR Vol.3 B.6.2.5/03

Table 26: Summary table of human data on serious eye damage/eye irritation

No data

Table 27: 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 performaed in accordance with GLP and OECD TG 405, 0.1 ml of tolclofos-methyl was placed into the conjunctival sac of the right eye of each three male rabbits (New Zealand White). The lids were then gently held together for one second to prevent loss of the test material. The left eye remained untreated and served as negative control. The treated eyes remained unwashed after application. All animals were observed daily for clinical signs during the experimental period. Ocular lesions were observed 1, 24, 48, 72 and 92 hours after application. The grading and scoring of irritation reactions were performed according to the method of Draize scale. A hand slit-lamp was used to score irritation reactions. After recording the observations at 24, 48, 72 and 96 hours, fluorescein stain was used to further examine the eyes. The irritation potential of the test substance was classified according to the method of Kay and Calandra. Redness (grade 1) and chemosis (grade 1) in all 3 rabbits were observed 1 hour after application. Twenty-four hours after application, redness (grade 1) in the conjunctiva in all 3 rabbits and chemosis (grade 2) and discharge (grade 1) in the conjunctiva in 1 out of 3 rabbits were observed. Forty-eight hours after application, redness (grade 1), chemosis (grade 1) and discharge (grade 1) in 1 out of 3 rabbits were observed. Seventy-two hours after application, redness (grade 1) and chemosis (grade 1) in 1 out of 3 rabbits were observed. These reactions disappeared 96 hours after application. The mean total scores (MTSs) of the irritation were calculated from the above results at each observation time point and the maximum mean total score (MMTS) of irritation was 4.0 at 1 and 24 hours after application. Mean irritation scores for conjunctival redness were: 1, 0.33, 0.33, Mean irritation scores for conjunctivae chemosis were: 1.33, 0, 0.33. Mean irritation scores for cornea and iris were: 0 (RAR Vol. 3, B.6.2.5/03).

Animals No.	Tissue	Reaction	Irritation score					Mean irritation score (24, 48, 72 h)
[h] after application			1	24	48	72	96	
1	Cornea		0	0	0	0	0	0
	Iris		0	0	0	0	0	0
	Conjunctiva	Redness	1	1	1	1	0	1
	Conjunctiva	Chemosis	1	2	1	1	0	1.33
	Conjunctiva	Discharge	0	1	1	0	0	Not relevant
2	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	Redness	1	1	0	0	0	0.33
	Conjunctiva	Chemosis	1	0	0	0	0	0
	Conjunctiva	Discharge	0	0	0	0	0	Not relevant
3	Cornea	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0
	Conjunctiva	Redness	1	1	0	0	0	0.33
	Conjunctiva	Chemosis	1	0	0	0	0	0
	Conjunctiva	Discharge	0	0	0	0	0	Not relevant

In a study (no-GLP, in house method), 50 mg tolclofos-methyl was applied to the conjunctival sac of one eye of eight male rabbits for either 5 minutes (group I) or 24 hours (group II). After application, eyes were washed with 300 ml of physiological saline for 2 minutes. The untreated eye served as a control. The examination of eye reactions was conducted 1, 24, 48 and 72 hours after application. Mean irritation scores for cornea, iris and conjunctiva hypermia and oedema was 0 (RAR Vol. 3, B.6.2.5/01).

Group	No. of animals	Tissue	Intensity of irritant reactions				
I	5		Time after treatment				
			1 hr	24 hrs	48 hrs	72 hrs	7 days
		Cornea	0	0	0	0	0
		Iris	0	0	0	0	0
		Conjunctiva hypermia	0	0	0	0	0
		Conjunctiva edema	0	0	0	0	0
II	3	Cornea	0	0	0	0	0
		Iris	0	0	0	0	0
		Conjunctiva hyperemia	0	0	0	0	0
		Conjunctiva edema	0	0	0	0	0

In another study (no-GLP, in house method), 0.1 mL of tolclofos-methyl was applied into the conjunctival sac of one eye of six female rabbits (New Zealand White) and examinations were made 1 hour and 1, 2, 3, 4 and 7 days after application. No corneal damage or iridial inflammation was observed in any animal. In all animals, transient mild conjunctival reactions with slight to moderate discharge were observed after one hour. These reactions had disappeared 24 and 48 hours after application in all animals. The mean score for conjunctivae redness is 0.06, the mean score for cornea, iris, conjunctivae chemosis and discharge is 0.0 (RAR Vol. 3, B.6.2.5/02).

	Cornea						**Iris**						**Conjunctivae**																	
													Redness						**Chemosis**						**Discharge**					
time/rabbit	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1 hour	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1
Day 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mean score Day 1-3	0.0						0.0						0.06						0.0						0.0					

10.5.2 Comparison with the CLP criteria

According to the CLP Guidance 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

-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"

In the eye irritation test conducted with tolclofos-methyl mean scores for corneal opacity did not exceed 1, and mean scores for conjunctival oedema or redness did not exceed 2. Furthermore, no effects were noted in iris and no effects persisted to the end of the observation period. Thus, tolclofos-methyl does not fulfil the classification criteria for eye irritation.

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

No classification is proposed for tolclofos-methyl

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Three rabbit eye irritation studies were included in the CLH dossier.

In a GLP and OECD 405 compliant study, 0.1 mL of tolclofos-methyl was placed into the conjunctival sac of the right eye of each of three male New Zealand White rabbits (Anon. 2010, RAR Vol.3 B.6.2.5/03). The treated eyes remained unwashed after application. No effects on the cornea or iris were observed in any animal, but all three showed conjunctival redness (grade 1) and chemosis (grade 1) one hour after application. In two animals, only redness (grade 1) persisted up to 24 hours, thereafter it had disappeared. In the third rabbit, redness (grade 1) was seen up to 72 hours. This rabbit also developed chemosis (grade 2) and discharge (grade 1) in the conjunctiva after 24 hours. Chemosis (grade 1) was also observed after 48 and 72 hours, discharge (grade 1) after 48 hours. After 96 hours, all symptoms had disappeared in this rabbit. Mean individual irritation scores over 24-72 hours were 1, 0.33 and 0.33 for conjunctival redness and 1.33, 0 and 0 for conjunctival chemosis. For the cornea and iris, all mean individual scores were 0.

In a non-GLP study using an in-house (OECD 405-like) method (Anon. 1978, RAR Vol.3 B.6.2.5/01), all scores for cornea, iris, conjunctival hyperaemia and conjunctival oedema, during the 7 days observation period, were 0 after instillation of 50 mg tolclofos-methyl in the eyes of male rabbits of native Japanese strain for either 5 min (n=5) or 24 hours (n=3).

Another non-GLP study also used an in-house (OECD 405-like) method, with application

of 0.1 mL tolclofos-methyl into the eyes of six female New Zealand White rabbits (Anon. 1985, RAR Vol.3 B.6.2.5/02). No effects on the cornea or iris were observed in any animal at any time-point. All animals displayed transient mild conjunctival reactions with slight to moderate discharge after one hour. These reactions had disappeared at 24 (n=5) or 48 hours (n=1) after application. For one animal the mean individual scores over 24-72 hours were 0.33 for conjunctival redness and 0 for cornea, iris and conjunctival chemosis and discharge. For the other five animals all mean individual scores over 24-72 hours were 0.

The DS proposed no classification for eye damage/irritation, given that in all three studies the mean scores for conjunctival oedema or redness did not exceed 2, no effects were noted in the iris and cornea, and no effects persisted to the end of the observation period.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Tolclofos-methyl induced relatively mild symptoms of eye irritation in two out of three available eye irritation studies in rabbits. The criteria for Category 2 include substances that produce in at least 2 of 3 tested animals a positive response of:

(a) corneal opacity ≥ 1; and/or

(b) iritis ≥ 1; and/or

(c) conjunctival redness ≥ 2; and/or

(d) conjunctival oedema (chemosis) ≥ 2,

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

As the findings in both positive studies remained below the limits of the classification criteria and were reversible, RAC agrees with the DS that **no classification is warranted for eye damage/irritation**.

10.6 Respiratory sensitisation

Table 28: Summary table of animal studies on respiratory sensitisation

No data

Table 29: Summary table of human data on respiratory sensitisation

No data

Table 30: Summary table of other studies relevant for respiratory sensitisation

No data

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

No data

10.6.2 Comparison with the CLP criteria

No data are available. The substance does not meet the criteria for classification for respiratory sensitisation

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification is proposed for tolclofos-methyl

10.7 Skin sensitisation

Table 31: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Buehler method In compliance to 92/69/EEC. B.6 *The study is limited. The results of the preliminary investigations were not reported. Unnecessary many inductions were performed. Few animals used in the treatment group* No GLP statement but QA	Guinea pig Hartley/Dunkin Treatment group: 10 females Control group: 10 females	Tolclofos-methyl (purity: 97.7%) Vehicle: acetone	Induction: 0.5 mL of a 50% w/w solution of tolclofos-methyl in acetone (nine induction applications each for 6 hours, three times a week during a three week period) Challenge: 50% w/w in acetone (topically two weeks after the last induction treatment) Skin reactions were evaluated 24, 48 and 72 hours after patch removal.	After several applications of tolclofos-methyl during induction, some irritation was noted on the induction site in the test group; no irritation occurred with the vehicle in the control group. After challenge, no dermal reactions were seen in any of the test or control animals.	RAR Vol. 3 B.6.2.6/01
Guinea Pig Maximisation Test	Guinea pig Hartley Treatment	Tolclofos-methyl (purity:	Induction: intradermal injection (0.1 mL) of 5% tolclofos-methyl	Slight to moderate erythema and slight to moderate swelling was observed in animals challenged with the test	RAR Vol. 3 B.6.2.6/02

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD TG 406 GLP: Yes	group: 20 females Control group: 10 females	98.0%) Vehicle: corn oil (1st induction) acetone (2nd induction and challenge)	in corn oil and Freund´s complete adjuvant/distilled water. One week after injection a patch containing 0.4 mL of test material (25% w/w in acetone) was placed on the skin area of sensitised animals for 48 hours under an occlusive dressing Challenge: Two weeks after the second induction 0.2 ml of the test material (10% in acetone) for 24 hours under an occlusive dressing. Observations were made 24 and 48 hrs after patch removal	material (sensitisation rate 35%).	

Table 32: Summary table of human data on skin sensitisation

No data

Table 33: 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 tolclofos-methyl was assessed in two studies in the guinea pig (Buehler method and Guinea Pig Maximisation Test (GPMT). Tolclofos-methyl was found to be positive in the GPMT. The Buehler study was negative but the study was considered limited (few animals were used and the results of the preliminary investigations were not reported).

In the skin sensitisation test according to GPMT, induction of 20 female guinea pigs (Hartley) was performed by intradermal injection (0.1 mL) of 5% tolclofos-methyl in corn oil and Freund´s complete adjuvant/distilled water. One week after injection a patch containing 0.4 mL of test material (25% w/w in acetone) was placed on the skin area of sensitised animals for 48 hours under an occlusive dressing. α-hexylcinnamaldehyde (HCA) was applied as the positive control material. Ten control animals were similarly treated with acetone and patch free of test material. Challenge treatment was performed two weeks after the second induction by using a patch containing 0.2 ml of the test material (10% in acetone) or positive control material for 24 hours under an occlusive dressing. Observations were made 24 and 48 hours after patch removal. Slight to moderate erythema was observed in 7 out of 20 animals and slight to moderate swelling was observed in 5 out of 20 animals in the test material sensitised group challenged with the test material at 10%. In the control group, skin reactions were not observed in any 10 animals. From these results, the sensitisation rate was estimated to be 35% (positive animals/all tested animals = 7/20). The

positive control group showed distinct sensitising reactions (sensitisation rate 100%) (RAR Vol. 3, B.6.2.6/02).

Group		Tolclofos-methyl sensitized				Tolclofos-methyl control				HCA sensitized				HCA control			
Material used for the induction treatment		tolclofos-methyl				-				HCA				-			
Material used for challenge treatment		tolclofos-methyl				tolclofos-methyl				HCA				HCA			
Concentration (%)		10				10				10				10			
Number of animals used		20				10				5				5			
Observation time		24 hrs		48 hrs		24 hrs		48 hrs		24 hrs		48 hrs		24 hrs		48 hrs	
Skin reaction*		E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
Grade**	0	13	16	13	15	10	10	10	10	0	1	0	1	5	5	5	5
	1	5	3	5	3	0	0	0	0	2	3	4	3	0	0	0	0
	2	2	1	2	2	0	0	0	0	3	1	1	1	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

In the Buhler test, ten female guinea-pigs (Hartley/Dunkin) received topical applications of 0.5 mL of a 50% (w/w) solution of tolclofos-methyl in acetone on patches placed on the clipped skin. Nine induction applications each for 6 hours were made three times a week during a three week period. The control group (10 female animals) was treated similarly, but without test material. Test and control animals (10 animals/group) were challenged with the test material (50% w/w in acetone) topically two weeks after the last induction treatment. Skin reactions were evaluated 24, 48 and 72 hours after patch removal. After several applications of tolclofos-methyl during induction, some irritation was noted on the induction site in the test group; no irritation occurred with the vehicle in the control group. After challenge, no dermal reactions were seen in any of the test or control animals. The study was considered limited (RAR Vol. 3, B.6.2.6/01).

10.7.2 Comparison with the CLP criteria

According to CLP Regulation 3.4.2.2.4, a response of at least 30% of the animals is considered as positive when an adjuvant type guinea pig test method for skin sensitisation is used. In the study performed with tolclofos-methyl where Guinea Pig Maximisation Test was used, the induction concentration of 5 % (first induction) and 25% (second induction) resulted in a sensitisation rate of 35% after a challenge with 10% test substance. Thus, tolclofos-methyl fulfils the criteria and should be classified as a skin sensitiser.

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. Classification into sub-categories is only allowed if data are sufficient (CLP Annex I, 3.4.2.2.1.1). Therefore care should be taken when classifying substances into Category 1B when Category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent (in line with some test protocols where a maximised dose should be used). The criteria for sub-categorisation based on results from Guinea Pig Maximisation Tests and Buehler assays are given in table below:

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
1A	Buehler assay	≥15% responding at ≤0.2% topical induction dose or ≥60% responding at >0.2% to ≤20% topical 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
1B	Buehler assay	≥15% to <60% responding at >0.2% to ≤20% topical induction dose or ≥15% responding at >20% topical induction dose

According to table above, tolclofos-methyl fulfils the criteria for subcategorisation in category 1B (Guinea Pig Maximisation Test: ≥30% responding at >1% intradermal induction dose.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Tolclofos-methyl fulfils the criteria for subcategorisation in category 1B (Guinea Pig Maximisation Test: ≥30% responding at >1% intradermal induction dose).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The CLH dossier included two skin sensitisation assays, one Buehler test and one Guinea Pig Maximisation Test (GPMT).

In the non-GLP, OECD 406-like Buehler test (Anon. 1985, RAR Vol.3 B.6.2.6/01), female Hartley/Dunkin guinea-pigs received topical applications (nine in total: each for 6 hours, 3 times a week for 3 weeks) of 0.5 mL of either a 50% (w/w) solution of tolclofos-methyl in acetone or the solvent only on patches placed on the clipped skin. Test and control animals (10 animals/group) were challenged with the test material (50% w/w in acetone) topically two weeks after the last induction treatment. The test concentrations were chosen based on preliminary investigations. Some irritation (no further details reported) was noted on the induction site in the test group; no irritation occurred with the vehicle in the control group. After challenge, no dermal reactions were seen in any of the test or control animals. The DS considered this study, however, to be limited, given that only 10 animals were used (where OECD 406 recommends 20), the results of the preliminary testing were not reported, and an unnecessary number of inductions had been performed (the test guideline recommends induction during 3 days only).

In the GLP and OECD 406 compliant GPMT (Anon. 2001, RAR Vol.3 B.6.2.6/02), female Hartley guinea pigs were induced by intradermal injection (0.1 mL) of 5% tolclofos-methyl in corn oil and Freund´s complete adjuvant/distilled water, followed one week

later by epidermal application of 25% w/w tolclofos-methyl in acetone (0.4 mL). The animals were challenged two weeks later with occlusive patch testing with a 10% tolclofos-methyl solution in acetone (0.2 mL). The test concentrations were chosen based on preliminary investigations. Slight to moderate erythema was observed in 7 out of 20 test animals (35%) and slight to moderate swelling in 5 out of 20 test animals. The sensitisation rates were 0% in the control group (n=10) and 100% in the positive hexylcinnamaldehyde (HCA) control group (n=5).

With a sensitisation rate of 35% after intradermal induction with 5% tolclofos-methyl in the GPMT, the DS concluded that tolclofos-methyl fulfils the criteria for a Category 1B skin sensitiser (≥ 30% response at > 1% intradermal induction dose). Hence, the DS proposed Skin Sens. 1B; H317.

Comments received during public consultation

One MSCA supported the classification proposal of the DS.

Assessment and comparison with the classification criteria

Tolclofos-methyl is currently classified in Cat. 1 for skin sensitisation, without specifying a subcategory. If sufficient information is available to determine the potency of a substance, it is generally preferred to classify in a specific subcategory.

Of the two available skin sensitisation studies, the negative Buehler test is considered limited due to its shortcomings. The GPMT used an induction concentration of 5% (first induction) and 25% (second induction), and resulted in a sensitisation rate of 35% after a challenge with 10% test substance. Hence, tolclofos-methyl is to be considered a skin sensitiser (≥ 30% response in an adjuvant type guinea pig study). As to the subcategory, the classification limits for the GPMT are:

Cat. 1A: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose;

Cat. 1B: ≥ 30% to < 60% responding at > 0.1 % to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose.

The response of 35% at an intradermal induction concentration of 5% fulfils the criteria for Cat. 1B. It should be noted that lower than 5% intradermal induction concentrations were not tested in the GPMT, so information on concentrations below 1% (the cut-off for Cat. 1A) is not available. However, considering that the response rate was only slightly above 30% after intradermal induction with a 5% solution, RAC considers it highly unlikely that intradermal induction concentrations below 0.1% would still give a response ≥ 30%, or intradermal induction concentrations between 0.1 and 1% a response ≥ 60%. For this reason, Cat. 1A can be excluded.

RAC thus agrees with the DS that tolclofos-methyl should be classified as **Skin Sens. 1B; H317**.

10.8 Germ cell mutagenicity

Table 34: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro mutagenicity testing of Tolclofos-methyl in the *Salmonella typhimurium* plate incorporation assay OECD 471 with some deviations; the plates is just in duplicate. 2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9-mix. If 2-aminoanthracene is used, each batch of S9 should also be characterised with a mutagen that requires metabolic activation by microsomal enzymes, e.g., benzo(a)pyrene, dimethylbenzanthracene. Also the other positive controls are not chosen in accordance with the guidance suggestions. GLP: No	Tolclofos-methyl Purity: 98.7 %	0, 10, 50, 100, 500, 1000, 5000 µg/plate for each tester strain in the presence and absence of S9 mix. Tester strains: *Salmonella typhimurium* TA 100, TA, 1535, TA 1537, TA 1538, TA 98 *Escherichia coli* WP2uvrA	The test chemical induced no increases in the number of revertant colonies of any strain at any dose. Tolclofos-methyl was non-mutagenic	RAR Vol. 3 B.6.4.1/01
In vitro mutagenicity testing of Tolclofos-methyl in the *Salmonella typhimurium* plate incorporation assay OECD 471 with some deviations; has not been possible to detect any cross-linking mutagen effect as neither *E.coli WP2 uvrA* nor *S. typhimurium TA120* was included in the reverse mutation test. GLP: No	Tolclofos-methyl Purity: 97 %	0, 10, 100, 500, 1000, 2000 µg/plate for each tester strain in the presence and absence of S9 mix	The number of revertant colonies appearing on the plates treated with tolclofos-methyl was similar to that of the negative controls, in the presence or absence of mammalian metabolizing enzymes. Tolclofos-methyl did not show any mutagenic potential under the test conditions.	RAR Vol. 3 B.6.4.1/02
In vitro chromosomal aberration test of Rizolex in Chinese hamster ovary cells. OECD 473 with some deviations; exposure time, 200 and not 300 cells scored. GLP: Yes	Tolclofos-methyl Purity: 96.6 %	0, 10 20 40 µg/ml (without metabolic activation), 37.5, 75, 150 (with metabolic activation)	Tolclofos-methyl did not induce chromosomal aberrations in CHO-K1 cells under the conditions used.	RAR Vol. 3 B.6.4.1/03
Mutation test: Chinese hamster lung cells (V79) and Unscheduled DNA synthesis: HeLa cell	Tolclofos-methyl Purity: not specified	Mutation test, V79 cells: 5×10^{-6}, 5×10^{-7}, 5×10^{-8} and 5×10^{-9} M with and without metabolic activation	Tolclofos-methyl did not induce gene mutation or unscheduled DNA synthesis in mammalian cells *in vitro*.	RAR Vol. 3 B.6.4.1/04

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD 476 and 482 *Deviation from OECD 476: Other positive controls was used compared to the guideline recommendation* GLP: No		Unscheduled DNA synthesis: 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M with and without metabolic activation		
In vitro unscheduled DNA synthesis (UDS) assay in rat hepatocytes. OECD 482 GLP: Yes	Tolclofos-methyl (Risolex) Purity: 96.6 %	0.3, 1.3, 10 20, 40 µg/ml	Tolclofos-methyl did not induce UDS and has no DNA-damaging activity on rat hepatocyte primary cultures.	RAR Vol. 3 B.6.4.1/05
In vivo chromosomal aberration test on bone marrow cells of mice. *OECD 475 deviation; only 4 animals was used in the positive control groups and not 5 as suggested in the guideline.* GLP: No	Tolclofos-methyl (S-3349) Purity: 99.8 %	1000, 2000 and 4000 mg/kg bw	Tolclofos-methyl is not clastogenic in this *in vivo* cytogenetic assay	RAR Vol. 3 B.6.4.2/01
Micronucleus test in mice. OECD 474 GLP: Yes	Tolclofos-methyl, technical grade Purity: 97.5 %	500, 1000 and 2000 mg/kg bw	Tolclofos-methyl does not induce micronuclei in mouse bone marrow cells	RAR Vol. 3 B.6.4.2/02
Mutagenicity evaluation of S-3349 T.G. Lot No. 4 in the rat dominant lethal assay. OECD 478 GLP: No	Tolclofos-methyl Purity: not specified	62.5, 208.3, 625 mg/kg bw/day	No reduction in fertility. No change in implants. No effect on corpora lutea. No effect on preimplantation loss. No dominant lethal effects induced by tolclofos-methyl under the conditions used in the study.	RAR Vol. 3 B.6.4.2/03

Table 35: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

No data

Table 36: 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 genotoxic studies are thoroughly presented in Vol. 3 to the RAR.

The genotoxic potential of tolclofos-methyl was investigated in five standard *in vitro* test systems (two Ames test, mammalian chromosome aberration test, mammalian cell gene mutation test and two unscheduled DNA tests) reinforced with a chromosome aberration study in mice bone marrow cells, and an *in vivo* mouse micronucleus test. The results from the guideline studies were consistently negative and based on these data, it was concluded that tolclofos-methyl does not possess any mutagenic or clastogenic properties. All these studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981).

The results of the *in vivo* mouse micronucleus test is summarised below:

Sampling time hr	Substance	Dose mg/kg	No. of mice	MNPCE/PCE (%)		PCE/(PCE+NCE) (%)	
				Mean	SKC	Mean	SW
24	Vehicle (Corn oil)	0	5	0.33	-	52.4	-
	Tolclofos-methyl T.G.	500	5	0.23	N.S.	52.7	N.S.
		1000	5	0.23	N.S.	55.7	N.S.
		2000	5	0.23	N.S.	57.2	N.S.
	Mitomycin C	0.5	5	2.73	***	54.6	N.S.
48	Vehicle (Corn oil)	0	5	0.23	-	54.8	-
	Tolclofos-methyl T.G.	2000	5	0.17	N.S.	45.2	*

Testing using germ cells was not triggered as all genotoxicity studies (above) were negative and no evidence for carcinogenicity was observed in long-term experiments in the rat and mouse. Anyhow a mutagenicity evaluation in rat dominant lethal assay was performed and found negative. No classification is necessary with regard to genotoxicity.

No photomutagenicity study is required as the molar extinction/absorption coefficient between 290 and 700 nm is below 1000 L x mol^{-1} x cm^{-1}. In addition, tolclofos-methyl has been shown to be negative in a standard *in vitro* phototoxicity study, thus no photosafety concern exists.

10.8.2 Comparison with the CLP criteria

The CLP Regulation allows classification of genotoxicity substances in two hazard categories, Category 1 and 2. Category 1 comprises two sub-categories, 1A and 1B. The criteria for the categories and sub-categorisation are given in table below:

Sub-category	Assay
Category 1	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B	The classification in Category 1B is based on: -positive results(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.
Category 2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: -positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: Somatic cell mutagenicity tests *in vivo*, in mammals; or Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note; Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

The results from the guideline studies performed with tolclofos-methyl were consistently negative. Thus, tolclofos-methyl does not fulfil the classification criteria for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is porposed for tolclofos-methyl

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro, tolclofos-methyl was tested in two Ames tests with *Salmonella typhimurium* and *Escherichia coli* (Moriya et al. 1981, RAR Vol.3 B.6.4.1/01; Suzuki & Miyamoto 1978, RAR Vol.3 B.6.4.1/02), a chromosomal aberration test in Chinese hamster ovary cells (Kogis 1990, RAR Vol.3 B.6.4.1/03; GLP), a gene mutation test in Chinese hamster lung cells (Monaco & Nunziata 1981, RAR Vol.3 B.6.4.1/04) and two unscheduled DNA synthesis tests, one in HeLa cells (Monaco & Nunziata 1981, RAR Vol.3 B.6.4.1/04) and the other in rat hepatocytes (Anon. 1990, RAR Vol.3 B.6.4.1/05; GLP).

The *in vivo* tests with tolclofos-methyl included a chromosomal aberration test (Anon. 1981, RAR Vol.3 B.6.4.2/01) and a micronucleus test (Anon. 2013, RAR Vol.3 B.6.4.2/02; GLP) on bone marrow cells in mice (at single doses up to 4000 (intraperitoneal) and 2000 (oral) mg/kg bw, respectively), and a dominant lethal assay (Anon. 1981, RAR Vol.3

B.6.4.2/03) in rats (at single oral doses up to 625 mg/kg bw).

All studies were basically conducted in accordance with OECD test guidelines. In all *in vitro* and *in vivo* studies, tolclofos-methyl tested negative under the conditions used. Given the consistent negative results, the DS proposed no classification for germ cell mutagenicity.

Comments received during public consultation

One comment from IND was received, concerning some editorial remarks on table 34 in the CLH report.

Assessment and comparison with the classification criteria

Several *in vitro* and *in vivo* mutagenicity and genotoxicity tests are available for tolclofos-methyl. The most relevant studies for the assessment of classification are the rat dominant lethal test for the detection of germ cell mutagenicity, and the somatic *in vivo* chromosomal aberration test and micronucleus assay, both performed in the bone marrow of mice. All three studies were negative. The *in vitro* assays in bacteria and in mammalian cells (investigating chromosomal aberrations, gene mutations and UDS) were also negative.

From the available studies tolclofos-methyl does not appear to have mutagenic or genotoxic properties. Therefore, RAC agrees with the DS that **no classification is warranted for germ cell mutagenicity**.

10.9 Carcinogenicity

Table 37: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined two year chronic toxicity/carcinogenicity study in rats (Fischer 344 CD®F) In house method The study was checked for compliance with OECD 453. Following deviation was noted: males were exposed up to 122 weeks and females up to 129 weeks GLP: No	Tolclofos-methyl, 94.9% 0, 100, 300, 1000 ppm equivalent to 0, 4.2, 12, 42 mg/kg bw/day (males) 0, 4.8, 15, 49 mg/kg bw/day (females)	No distinct signs of compound effect were observed with regard to mortality, clinical signs, body weights, food consumption, organ weights and organ/body weight ratios, gross pathology and histopathology were attributable to the test compound at dietary levels up to 1000 ppm. Minor changes in clinical chemistry parameters (↓alkaline phosphatase) were noted in males of all treated groups. The high dose, 1000 ppm, represent a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies. NOAEL: ≥ 1000 ppm (≥42 mg/kg bw/day) Tolclofos-methyl was not oncogenic in this study.	RAR Vol. 3 B.6.5.1/01
104-week cholinesterase activity study in male and female rats (Fischer 344) In house method GLP: No but performed according to GLP of US Food and Drug Administration *The high dose, 1000 ppm, represents a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies.* The study is regarded as supplementary data.	Tolclofos-methyl, 98.3% 0, 100, 300, 1000 ppm equivalent to 0, 4.1, 12, 42 mg/kg bw/day (males) 0, 4.8, 15, 49 mg/kg bw/day (females)	No treatment-related effects were shown on the plasma, erythrocyte or brain cholinesterase. NOAEL: ≥1000 ppm (equivalent to 42 mg/kg bw/day).	RAR Vol. 3 B.6.5.1/02
Combined two year chronic toxicity/carcinogenicity study in mice (Crj:B6C3F1) In house method Checked for compliance with OECD 453: Deviations: prothrombin time, activated partial thromboplastin time, albumin, calcium, sodium, potassium, total cholesterol were not measured GLP: No	Tolclofos-methyl, 94.3% 0, 10, 50, 250, 1000 ppm equivalent to 0, 1.3, 6.4, 32.2, 134 mg/kg bw/day (males) 0, 1.3, 6.9, 34.1, 137 mg/kg bw/day (females)	No treatment related mortality, no haematology changes. Suppression of weight gain after 52 weeks in the female 1000 ppm group (15%) and decreased food consumption at week 104 in the female 1000 group. Decrease in plasma, erythrocyte and brain cholinesterase at both sexes at 250 and 1000 ppm (At 250 ppm (week 104): plasma cholinesterase activity reduced 25% in males and 33% in females; erythrocyte cholinesterase activity reduced 13% in males and 11% in females, brain cholinesterase activity reduced 13% in males; At 1000 ppm: plasma cholinesterase activity reduced 43% in males and 59% in females, erythrocyte cholinesterase activity reduced 13%	RAR Vol. 3 B.6.5.2/01

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		in males and 23% in females, brain cholinesterase activity reduced 26% in males and 9% in females). At week 28 in females, the cholinesterase activity was decreased already at 50 ppm (reduction in plasma (12%) and brain (18%)). At week 52 erythrocyte cholinesterase activity was reduced 23% in females and 18% in males at 250 ppm, and brain cholinesterase activity was reduced 12% in females and 14% in males at 250 ppm Glucose was increased in male 1000 ppm group after 104 weeks. Increase in kidney weight noted in both sexes at ≥250 ppm (250 ppm (week 52): ↑absolute weight 15% (males) ↑relative weight 9% (females); 250 ppm (week 104): ↑absolute weight 3% (females), ↑relative weight 11% (females); 1000 ppm (week 52): ↑absolute weight 15% (males), ↑relative weight 11% (males) 14% (females); 1000 ppm (week 104): ↑absolute weight 3% (females), ↑relative weight 10% (females)) Decrease in thymus weight at 1000 ppm in females (At week 52: ↓absolute weight 31%, ↓rel weight 23%; At week 104: ↓absolute weight 18%) Increase in pituitary weight noted in females at 1000 ppm (At week 52: ↑ rel weight 23%, ↑absolute weight 31%; At week 104: ↑ rel weight 38%, ↑absolute weight 32%) NOAEL: 50 ppm, equivalent to 6.4 mg/kg bw/day based on decreased (more than 20 %) cholinesterase activity in erythrocytes and brain (noted at ≥ 250 ppm, equivalent to 32.2 mg/kg bw/day) and organ weight changes noted at ≥250 ppm Tolclofos-methyl did not show any carcinogenic activity in this study.	

Table 38: Summary table of human data on carcinogenicity

No data

Table 39: 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 two long-term studies in the rat (one combined chronic/carcinogenic toxicity test and a special two year test in order to study acetylcholinesterase activity) and one long-term toxicity study in the mouse. The long-term toxicity studies are thoroughly presented in Vo. 3 to the RAR.

In the combined chronic/carcinogenic toxicity test in the rat (no GLP) no signs of compound effect were observed with regard to mortality, clinical signs, body weights, food consumption, organ weights and organ/body weight ratios, and gross pathology. No histomorphological alterations were attributable to the test compound at dietary levels up to 1000 ppm. Tolclofos-methyl was not oncogenic in this study. The study is acceptable, however, it can be noted that the high dose, 1000 ppm, represent a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies. It is therefore not possible to set the precise NOAEL for the study; NOAEL: ≥ 1000 ppm (42 mg/kg bw/day) (RAR Vol. 3, B.6.5.1/01)

Chronic toxicity study in rats: Incidence of histopathologically proven tumors

Dose level	**0 ppm**	**100 ppm**	**300 ppm**	**1000 ppm**
Dead and moribund sacrifices				
No. examined	31	26	27	35
Interstitial cell tumors	29	24	26	34
Mesothalioma	1	2	0	2
Terminal sacrifices				
No. examined	23	28	27	19
Interstitial cell tumors	23	28	27	18
Mesothelioma	1	1	1	1

Chronic toxicity study in rats: Neoplasms classification summary (Dead and moribund sacrifices)

	Dead and moribund sacrifices							
Sex	Male				Female			
Dose level (ppm)	0	100	300	1000	0	100	300	1000
Neoplasm classification summary								
Number of animals	32	27	28	36	36	27	34	25
Total primary neoplasms	83	69	84	97	82	53	61	41
Animals with one or more	31	26	27	36	32	25	31	22
Percent with one or more	96 %	96 %	96 %	100 %	88 %	92 %	91 %	88 %
Total begnin neoplasms	51	45	54	66	44	28	37	19
Animals with one or more	30	25	27	36	26	18	23	14

Percent with one or more	93 %	92 %	96 %	100 %	72 %	66 %	67 %	56 %
Total malignant neoplasms	32	24	30	31	38	25	24	22
Animals with one or more	24	17	21	21	25	20	20	16
Percent with one or more	75 %	62 %	75 %	58 %	69 %	74 %	58 %	64 %
Total metastatic neoplasms	8	6	0	2	0	4	1	1
Animals with one or more	2	3	0	2	0	2	1	1
Percent with one or more	6 %	11 %	0 %	5 %	0 %	7 %	2 %	4 %
Total locally invasive neoplasms	2	0	2	1	2	6	3	2
Animals with one or more	2	0	1	1	2	4	2	1
Percent with one or more	6 %	0 %	3 %	2 %	5 %	14 %	5 %	4 %
Total other neoplasms	0	0	0	0	0	0	0	0
Animals with one or more	0	0	0	0	0	0	0	0
Percent with one or more	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

Chronic toxicity study in rats: Neoplasms classification summary (Terminal sacrifices)

	Terminal sacrifices							
Sex	Male				Female			
Dose level (ppm)	0	100	300	1000	0	100	300	1000
Neoplasm classification summary								
Number of animals	23	28	27	19	19	28	21	30
Total primary neoplasms	80	86	101	60	67	92	60	93
Animals with one or more	23	28	27	19	19	25	21	30
Percent with one or more	100 %	100 %	100 %	100 %	100 %	89 %	100 %	100 %
Total begnin neoplasms	54	60	67	44	41	50	38	62
Animals with one or more	23	28	27	19	18	23	19	28
Percent with one or more	100 %	100 %	100 %	100 %	94 %	82 %	90 %	93 %
Total malignant neoplasms	26	26	34	16	26	42	22	31
Animals with one or more	19	17	23	11	18	24	16	22
Percent with one or more	82 %	60 %	85 %	57 %	94 %	85 %	76 %	73 %
Total metastatic neoplasms	8	4	0	4	1	8	1	0

Animals with one or more	2	1	0	2	1	1	1	0
Percent with one or more	8 %	3 %	0 %	10 %	5 %	3 %	4 %	0 %
Total locally invasive neoplasms	0	0	0	0	1	0	1	0
Animals with one or more	0	0	0	0	1	0	1	0
Percent with one or more	0 %	0 %	0 %	0 %	5 %	0 %	4 %	0 %
Total other neoplasms	0	0	0	0	0	0	0	0
Animals with one or more	0	0	0	0	0	0	0	0
Percent with one or more	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

The assessment of cholinesterase activity was assessed in a second long-term toxicity study in the rat. In this study samples were taken from 10 animals/sex/group at initiation of the study, at weeks 5, 14, 27, 53, 79 and at termination for blood cholinesterase determination; for brain cholinesterase activity determination, samples were taken at week 53 and at termination. Food consumption and body weights were measured weekly during the first 26 weeks, once every two weeks from weeks 26 through 52 and once every four weeks from weeks 53 through 104. Examination and palpation for incidence and location of tissue masses were performed at each weighing interval. All animals were subjected to gross pathology at necropsy. There were no compound related effect observed with regard to mortality, clinical signs, body weights, food consumption and gross pathology. Mean erythrocyte cholinesterase and brain cholinesterase activities were comparable among groups throughout the study. NOAEL: ≥1000 ppm (equivalent to 42 mg/kg bw/day). The study is regarded as supplementary data only (RAR Vol. 3, B.6.5.1/02)

Dose level	0 ppm	100 ppm	300 ppm	1000 ppm
Males				
Erythrocyte cholinesterase activity (MCM/ML)[a]				
Week 0	6.1	6.1	6.0	6.1
Week 5	5.8	5.7	5.6	5.5
Week 14	5.8	5.7	5.7	7.6[b]
Week 27	6.1	6.5	6.1	6.3
Week 53	5.8	5.8	5.8	5.7
Week 79	6.2	6.6	6.2	6.1
Terminal	6.2	5.9	6.5	5.9
Plasma cholinesterase activity (MCM/ML)[a]				
Week 0	3.3	3.4	3.2	3.3
Week 5	2.7	2.8	2.7	2.6
Week 14	2.7	2.7	2.7	2.5
Week 27	3.2	3.1	2.9*	2.7*
Week 53	3.6	3.7	3.1	3.3
Week 79	4.9	4.6	4.4	4.2
Terminal	7.6	6.1	6.5	5.4

Brain cholinesterase activity (MCM/ML)[a]				
Week 53	52.8	50.6	50.0	49.1
Terminal	54.3	53.1	51.3	52.3
Females				
Erythrocyte cholinesterase activity (MCM/ML)[a]				
Week 0	5.8	6.0	5.8	5.8
Week 5	6.2	6.0	6.0	6.1
Week 14	6.0	5.8	5.7	6.1
Week 27	6.7	6.4	6.1	6.1
Week 53	5.4	5.2	5.0	5.1
Week 79	6.0	6.0	6.4	6.3
Terminal	6.1	6.8	6.1	6.5
Plasma cholinesterase activity (MCM/ML)[a]				
Week 0	5.6	4.8*	5.2	5.2
Week 5	8.6	8.8	8.3	7.7
Week 14	12.9	13.1	12.4	10.8* (16%)
Week 27	14.2	16.6	13.7	12.9
Week 53	14.9	16.8	14.0	14.0
Week 79	11.0	13.0	12.8	11.4
Terminal	11.2	12.0	13.3	11.1
Brain cholinesterase activity (MCM/ML)[b]				
Week 53	51.7	49.5	51.1	49.3
Terminal	52.9	54.6	53.5	53.9

a: Micromoles of sulfhydryl groups liberated in three minutes from 1 ml of sample
b: Excluding the three artefactually elevated values the mean is 5.8.
*: Significantly different from control ($p < 0.05$)

In the long-term toxicity study in the mouse (no GLP) treatment with tolclofos-methyl was associated with reduced bodyweight gain after 52 weeks in the female 1000 ppm group (15%) and decreased food consumption at week 104 in the female 1000 group. Decrease in plasma, erythrocyte and brain cholinesterase was noted in both sexes at 250 and 1000 ppm (At 250 ppm (week 104): plasma cholinesterase activity reduced 25% in males and 33% in females; erythrocyte cholinesterase activity reduced 13% in males and 11% in females, brain cholinesterase activity reduced 13% in males; At 1000 ppm: plasma cholinesterase activity reduced 43% in males and 59% in females, erythrocyte cholinesterase activity reduced 13% in males and 23% in females, brain cholinesterase activity reduced 26% in males and 9% in females). At week 28 in females, the cholinesterase activity was decreased already at 50 ppm (reduction in plasma (12%) and brain (18%)). At week 52 erythrocyte cholinesterase activity was reduced 23% in females and 18% in males, and brain cholinesterase activity was reduced 12% in females and 14% in males. Furthermore increased glucose was noted in male 1000 ppm group after 104 weeks. Organ weight changes were noted in both sexes at 250 ppm and above. Increase in kidney weights were noted in both sexes at ≥250 ppm, reduced thymus weight was noted at 1000 ppm in females and increased pituitary weight was noted in females at 1000 ppm. Tolclofos-methyl did not show any carcinogenic activity in this study (RAR Vol. 3, B.6.5.2).

Dose level	**0 ppm**	**10 ppm**	**50 ppm**	**250 ppm**	**1000 ppm**
Males					
Cholinesterase activity (µmol/ml/min)					
Serum					
Week 28	6.23	6.06	5.62	3.69*** (41%)	1.83*** (71%)
Week 52	6.33	6.07	6.05	4.27*** (32%)	2.92*** (54%)
Week 104	8.89	8.89	8.42	6.66*** (25%)	5.06*** (43%)
Erythrocytes					
Week 28	5.49	5.33	5.60	4.03*** (26%)	3.88*** (29%)
Week 52	5.22	5.13	5.39	4.28** (18%)	3.67*** (30%)
Week 104	5.21	5.13	5.14	4.54* (13%)	4.53* (13%)
Brain					
Week 28	20.16	18.39	18.42	18.21	16.70
Week 52	18.85	18.43	19.00	16.20* (14%)	14.27*** (24%)
Week 104	17.77	17.83	16.59	15.41* (13%)	13.16*** (26%)
Glucose (mg/dl)					
Week 52	117.13	107.50	110.63	105.00	107.51
Week 104	122.27	114.18	127.86	128.29	140.76* (115%)
Females					
Cholinesterase activity (µmol/ml/min)					
Serum					
Week 28	9.46	9.20	8.28*** (12%)	4.51*** (52%)	1.79*** (81%)
Week 52	8.51	8.33	7.56	4.83*** (43%)	2.68*** (69%)
Week 104	9.01	9.28	8.58	6.04*** (33%)	3.68*** (59%)
Erythrocytes					
Week 28	5.87	5.79	5.62	4.20*** (28%)	3.10*** (47%)
Week 52	5.13	4.97	5.10	3.96* (23%)	3.19*** (38%)

Week 104	5.25	5.08	5.11	4.67* (11%)	4.04*** (23%)
Brain					
Week 28	21.40	19.63	17.52* (18%)	16.32** (24%)	14.42*** (33%)
Week 52	18.77	17.83	19.86	16.45* (12%)	15.89** (15%)
Week 104	17.92	17.89	18.71	16.61	16.24* (9%)
Glucose (mg/dl)					
Week 52	111.66	109.80	109.92	107.13	103.80
Week 104	122.45	124.98	123.50	122.89	132.14

*: $p < 0.05$ in comparison with controls
**: $p < 0.01$ in comparison with controls
***: $p < 0.001$ in comparison with controls

10.9.2 Comparison with the CLP criteria

According to Regulation 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 tolclofos-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. […]"*

Tolclofos-methyl does not fulfil this criteria since no carcinogenic activity was noted in available long-term/carcinogenicity studies in rats and mice.

The placing of 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 (2) 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 organs"

Tolclofos-methyl does not fulfil this criteria since no carcinogenic activity was noted in available long-term/carcinogenicity studies in rats and mice.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification is proposed for tolclofos-methyl

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two 2-year studies (both non-GLP and using an in-house (OECD 453-like) method) were available to inform on the carcinogenic potential of tolclofos-methyl, one in rats and one in mice.

Rats

Groups of 55 Fischer 344 CD®F rats/sex were fed diets containing 0, 100, 300 or 1000 ppm tolclofos-methyl for 122 weeks (males; corresponding to mean intakes of 0, 4.2, 12 and 42 mg/kg bw/d) or 129 weeks (females; corresponding to mean intakes of 0, 4.8, 15 and 49 mg/kg bw/d) (Anon. 1985, RAR Vol.3 B.6.5.1/01). As no treatment-related increases in incidences of neoplastic lesions were observed, tolclofos-methyl was concluded to be not carcinogenic in rats. It was however noted that the high dose of 1000 ppm represents a low dose in terms of mg/kg bw/d and is in fact a non-toxic dose, given the absence of treatment-related effects on mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, urinalysis, acetylcholinesterase activity (in plasma, erythrocytes, brain), organ weights, gross pathology and histopathology.

Mice

Exposure of Crj:B6C3F1 mice (50/sex/group) to 0, 10, 50, 250 or 1000 ppm tolclofos-methyl in the diet for 24 months (corresponding to mean intakes of 0, 1.3, 6.4, 32.2 and 134 mg/kg bw/d for males and 0, 1.3, 6.9, 34.1 and 137 mg/kg bw/d for females) did not result in treatment-related increases in incidences of neoplastic lesions (Anon. 1983, RAR Vol.3 B.6.5.2/01). Tolclofos-methyl was therefore concluded to be not carcinogenic in mice. In contrast to rats, some toxicity was observed at the two high dose levels of 250 and 1000 ppm, including suppression of weight gain and food consumption in females at 1000 ppm, decrease in plasma, erythrocyte and brain acetylcholinesterase levels in both sexes at 250 and 1000 ppm, increase in glucose levels in males at 1000 ppm, increase in kidney weight in both sexes at 250 and 1000 ppm, decrease in thymus

weight and increase in pituitary weight in females at 1000 ppm.

In the absence of carcinogenic activity of tolclofos-methyl in the available chronic toxicity/carcinogenicity studies in rats and mice, the DS proposed no classification for carcinogenicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the available long-term studies in rats and mice no evidence for carcinogenicity of tolclofos-methyl was observed. RAC however notes that the study with rats does not inform fully on this property, as the dose levels were too low (no toxicity was seen up to and including the top dose, which was far below the limit of dose of 1000 mg/kg bw/d). It is also noteworthy that the number of animals with neoplasms in the rat study reached 100%, not only in the treatment groups but also in the control group. With such a high number in controls, any treatment-related effect would be very difficult to detect.

Noting the study shortcomings mentioned in the paragraph above, weighing the lack of genotoxicity of tolclofos-methyl and the absence of pre-neoplastic lesions in the long-term studies with rats and mice, RAC agrees with the DS that, overall, the available data **do not warrant classification for carcinogenicity**.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 40: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Three-generation Oral (dietary) In house method Rat Sprague Dawley CD albino M, F P1: 30/sex/group P2: 100 /sex/generation P3: 25/sex/group No GLP but a QA statement *Primordial follicles and sperm parameters, and sexual maturation were not investigated in study. prostate, seminal vesicles, pituitary, thyroid and uterus were not weighed. Histopathological examination was not performed for vaigna and epididymides. Dose levels were low.*	Tolclofos-methyl (purity: 98.7%) 0, 100, 300, 1000 ppm. Equivalent to (during 15 weeks pre-mating): P1: 0, 6.9, 20.5, 70.6 mg/kg bw/day in males; 0, 8.9, 26.2, 90.5 mg/kg bw/day in females P2: 0, 7.9, 23.4, 79.6 mg/kg bw/day in males; 0, 9.2, 26.9, 98.5 mg/kg bw/day in females P3: 0, 7.6, 23.8, 78.2 mg/kg bw/day in males; 0, 9.0, 28.4, 96.1 mg/kg bw/day in females 15 weeks prior to mating and during mating, gestation and lactation.	<u>100 ppm:</u> No treatment related effects (Findings considered incidental in nature and showed no trend that could be attributed to treatment) <u>300 ppm:</u> Parental: ↓bw in P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (Finding were considered incidental in nature and showed no consistent trend that could be attributed to the treatment) <u>1000 ppm:</u> Parental: ↓bw in P2 and P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (Findings were considered incidental in nature and showed no consistent trend that could be attributed to the treatment) NOAEL parental and pups: ≥1000 ppm (≥70.6 mg/kg bw/day) NOAEL reproductive: ≥1000 ppm (≥70.6 mg/kg bw/day)	RAR Vol. 3 B.6.6.1/01
One-generation Oral (dietary) In house method Rat Crj:CD(SD)(SPF) M, F P: 10/sex/group	Tolclofos-methyl (purity: 97.1%) 0, 2500, 5000, 10000 ppm. Equivalent to average intakes in the parental generation during pre-mating in males of 0, 173, 338 and 680 mg/kg bw/day and in females of 0, 178, 353, 668 mg/kg bw/day. For the pregnant and delivered dams average intakes were 0, 175, 341 and 642 mg/kg bw/day day during the gestational period,	Parental <u>2500 ppm:</u> -changes in organ weights (↑ abs liver (F: 8%)) <u>5000 ppm:</u> ↓bw (F: gestation period (Day 7: 5%), lactation period (5-9%)	RAR Vol. 3 B.6.6.1/02

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
F1: 10/sex/group GLP: No *Low number of animals in each group. Males treated 2 weeks prior to mating (OECD TG 415 recommends ten weeks prior to the mating period)*	and 0, 360, 698 and 1253 mg/kg bw/day during the lactation period. In the offspring generation average intakes were 0, 255, 519 and 1161 mg/kg bw/day in males and 0, 257, 529 and 1174 mg/kg bw/day in females. Parent females: from 2 weeks prior to mating until autopsy after weaning throughout mating, gestation and lactation periods. Parent males: from 2 weeks prior to mating to autopsy. F1 generation: from weaning to autopsy at the age of 8 weeks.	↓bw gain (F: lactation period 0-14 (43%)) ↓FC (F: middle to late stage of lactation period) ↓cholinesterase values in brain (M: 6%, F: 11%) -changes in organ weights (↑ abs liver (F:21%), (↑ rel liver (F:27%), ↑rel kidney (F:13%)) 10000 ppm: ↓bw (M: early premating period (Week 1: 5%); F: gestation period (5-7%), lactation period (8-20%) ↓bw gain (M: early premating period (Weeks 0-1: 33%), F: premating period (Weeks 0-1: 51%), lactation period (period 0-7: 84%, period 0-14: -8.6 g, period 0-21: -4.3 g) ↓FC (F: early premating, gestation, lactation) ↓cholinesterase values in brain (M: 11%, F: 18%) -organ weight changes (↑rel brain (F), ↑ abs liver (M:17%, F:25%), ↑ rel liver (M:21%, F:51%), ↑rel kidney (M:9%, F:18%), ↓abs and rel ovaries, ↓abs and rel uterus) Offsprings: 2500 ppm: ↓bw (F1 generation: M: 8% (PND 21) 9% (PND 28), F: 8% (PND 21 and 35) 7% (PND 56)) ↓bw gain (F1 generation: F: 14% (PND 21-49) ↓FC (M,F) -organ weight changes in F1 pups (↓abs spleen (M, F), ↓rel spleen (M)) -organ weight changes in F1 generation (↑rel liver (M: 9%), ↑rel kidney (F: 19%)) 5000 ppm: ↓bw (F1 pups: M: 15% (PND 21), F: 17% (PND 21); F1 generation: M: 10% (PND 56), F: 11% (PND 56))	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓bw gain (F1 pups: M: 16% (PND 0-21); F: 17% (PND 0-21); F1 generation: M: 9% (PND 21-56), F: 10% (PND 0-21)) ↓FC (M, F) -organ weight changes in F1 pups (↓abs brain (M), ↑ rel brain (M, F), ↓abs spleen (M, F) ,↓rel spleen (M, F)) -organ weight changes in F1 generation (↓abs brain (M, F), ↑rel liver (M:16%, F: 24%), ↑rel kidney (M:18%, F:21%), ↑rel testis) <u>10000 ppm:</u> ↓bw (F1 pups: M: 46%, F: 44%; F1 generation: M: 31% (PND 56); F: 24% (PND 56)) ↓bw gain (F1 pups: M: 50% (PND 0-21); F: 48% (PND 0-21); F1 generation: M: 27% (PND 21-56), F: 16% (PND 21-56)) ↓FC (M, F) -delayed preputial separation (preputial separation completed Day 46, compared to 43.6 in controls) -organ weight changes in F1 pups (↓abs brain (M, F), ↑ rel brain (M, F), ↓abs thymus (M, F), ↓rel thymus (M), ↓abs spleen (M, F), ↓rel spleen (M, F) ↓abs uterus -organ weight changes in F1 generation (↓abs brain (M, F), ↑ rel brain (M, F), ↑rel liver (M:39%, F:45%), ↓abs kidney (M:15%), ↑rel kidney (M:23%, F:22%), ↑rel testis, ↓abs ovary, ↓abs epididymides, ↑rel epididymides, ↓abs seminal vesicles, ↑rel prostate 25%) NOAEL parental: 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) NOAEL offspring generation: not established.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		NOAEL reproductive toxicity: ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively) LOAEL parental: 5000 ppm (338 and 353 mg/kg bw/day in males and females, respectively) LOAEL offspring generation: 2500 ppm (255 and 257 mg/kg bw/day in males and females) LOAEL reproductive toxicity: not estimated	

M: male
F: female

Table 41: Summary table of human data on adverse effects on sexual function and fertility

No data

Table 42: 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

The reproductive toxicity studies are thoroughly presented in Vol. 3 to the RAR.

In the three generation reproduction study in rats neither systemic toxic effects in the parental and in the pup generations nor a reprotoxic effect was observed when tested at dose levels up to 1000 ppm (70.6 mg/kg bw/day). The parental NOAEL was ≥1000 ppm (≥70.6 mg/kg bw/day). The NOAEL for pups and reproduction were ≥1000 ppm (≥70.6 mg/kg bw/day). The dose levels used in the study were low (RAR Vol. 3 B.6.6.1/01).

Tolclofos-methyl was further investigated in a newly submitted one generation reproductive study at doses up to 10000 ppm (680 and 668 mg/kg bw/day in males and females, respectively). Systemic toxicity for both the parental and the offspring generation comprised a reduction in body weights, bodyweight gains and food consumption, and increased liver and kidney weights noted in the parental animals at ≥5000 ppm (≥338 mg/kg bw/day) and in the offspring at ≥2500 ppm (≥255 mg/kg bw/day). Furthermore, the offspring generation showed an increased prostate weight (relative weight increased 25%) at 10000 ppm (1161 mg/kg bw/day). In addition to these findings, a decrease in brain cholinesterase activity (less than 20%) was observed in the parental animals at ≥5000 ppm. The reproductive performance was not affected by tolclofos-methyl treatment. A delay of starting and completing separation was noted in the offspring at 10000 ppm. This delay was considered a secondary effect due to the body weight suppression noted at this dose level. The histopathological examination of genital organs revealed no treatment related changes. For the parental generation, the NOAEL for systemic toxicity was 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) based on reduced bodyweight gain noted in females at ≥5000 ppm and in males at

10000 ppm, reduced body weight noted in females at 10000 ppm, and organ weight changes in the liver and kidneys noted at ≥5000 ppm. NOAEL for the offspring generation could not be derived. Reduced body weights and bodyweight gains and increased organ weights of liver and kidney were noted in all treated groups. In addition, the relative weight of prostate was increased (25%) at 10000 ppm. NOAEL for reproductive toxicity was set at ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively) (RAR Vol. 3, B.6.6.1/02).

Comment: The acceptability of the one generation study in rats and the parental reproductive and offspring NOAEL was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Experts´consultation 2.6). It was concluded that the one-generation study is considered acceptable and the parental, offspring and reproductive NOAELs proposed by RMS was agreed by the experts.

One generation reproduction study. Body weight (g) in males and females (P generation) during premating

Dose level (ppm)	**Premating period (weeks)**									
	0		**1**		**2**		**3**		**4**	
Male										
Male	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	324.6	13.0	371.1	19.8	403.4	24.6	427.0	26.4	456.7	30.7
2500	325.5	11.1	372.9	14.2	405.3	21.1	430.5	23.3	456.7	20.9
5000	323.1	7.8	365.3	10.5	398.1	14.5	426.0	14.6	455.3	16.0
10000	321.9	11.4	353.1* (5%)	14.0	388.7	18.4	408.3	21.2	442.1	22.2
Female										
0	210.9	6.3	225.3	7.5	233.7	8.6	261.5[1]	2.1	272.0[2]	0.0
2500	210.1	6.8	224.8	7.8	233.0	10.9	-		-	
5000	208.5	7.4	220.7	8.4	228.1	10.1	-		-	
10000	211.5	7.2	218.6	8.7	226.8	9.8	-		-	

*Significantly different from control group; p<0.05
1n=2
2n=1

One generation reproduction study. Body weight (g) in females (P generation) during gestation

Dose level (ppm)	**Gestation period (days)**																	
	0			**4**			**7**			**14**			**18**			**21**		
	Mean	SD	n	Mean	SD	n	Mean SD	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
0	240.8	13.8	9	263.7	10.8	9	275.0	10.2	9	307.0	10.0	9	349.0	15.2	9	392.2	21.3	9
2500	235.0	10.1	10	260.2	9.5	10	272.2	9.7	10	307.4	14.3	10	350.0	16.2	10	394.0	19.3	10
5000	232.2	11.2	10	252.2	12.4	10	262.4* (5%)	9.7	10	295.9	11.7	10	341.5	15.9	10	385.5	16.5	10
10000	228.0* (5%)	7.6	9	249.3* (5%)	9.6	9	260.6** (5%)	9.3	9	290.4* (5%)	8.8	9	330.4* (5%)	10.3	9	366.1* (7%)	12.9	9

Control group and 10000 ppm group: in each group one female animal was not pregnant and hence excluded: n=9
n: number of animals
*Significantly different from control group: p<0.05
**Significantly different from control group: p<0.01

One generation reproduction study. Body weight (g) in females (P generation) during lactation

Dose level (ppm)	**Lactation period (days)**														
	0			**4**			**7**			**14**			**21**		
	Mean	SD	n	Mean	SD	n	Mean SD	SD	n	Mean	SD	n	Mean	SD	n
0	287.1	12.2	9	304.1	11.5	9	310.2	10.6	9	319.8	11.5	9	310.8	10.9	9
2500	287.5	18.2	10	302.7	14.2	10	304.0	15.3	10	317.0	14.8	10	302.9	11.4	10
5000	273.5	16.5	10	284.4** (6%)	16.4	10	288.9** (7%)	14.1	10	292.3** (9%)	20.0	10	294.2* (5%)	14.8	10
10000	264.8* (8%)	14.1	9	271.3** (11%)	11.4	9	268.3** (14%)	12.0	9	256.2** (20%)	9.2	9	260.4** (16%)	15.3	9

Control group and 10000 ppm group: in each group one female animal was not pregnant and hence excluded: n=9
n: number of animals
*Significantly different from control group: p<0.05
**Significantly different from control group: p<0.01

One generation reproduction study. P-generation; Bodyweight gain (g) in males and females (premating period)

Dose level (ppm)	**Premating period (weeks)**							
	0-1		**0-2**		**0-3**		**0-4**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Males								
0	46.5	9.4	78.8	14.4	102.4	17.6	132.1	21.5
2500	47.4	5.6	79.8	15.0	105.0	17.0	131.2	15.9
5000	42.2	5.0	75.0	10.2	102.9	11.0	132.2	15.1
10000	31.2** (33%)	8.4	66.8	13.1	86.4	16.3	120.2	15.7
Females								
0	14.4	4.4	22.8	6.5	47.5[1]	4.9	55.5[1]	0.7
2500	14.7	6.7	22.9	9.1	-		-	
5000	12.2	3.6	19.6	6.6	-		-	
10000	7.1* (51%)	7.5	15.3	8.5	-		-	

*Significantly different from control group: p<0.05
**Significantly different from control group: p<0.01
[1]n=2

One generation reproduction study. P-generation; Bodyweight gain (g) in females (gestation period)

Dose level (ppm)	**Gestation period (days)**									
	0-4		**0-7**		**0-14**		**0-18**		**0-21**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	22.9[a]	5.7	34.2[a]	8.1	66.2[a]	13.0	108.2[a]	15.0	151.4[a]	20.7
2500	25.2	4.8	37.2	5.7	72.4	10.6	115.0	15.6	159.0	18.5
5000	20.0	4.3	30.2	5.0	63.7	7.8	109.3	8.7	153.3	9.6
10000	21.3[a]	5.9	32.6[a]	4.4	62.4[a]	6.9	102.4[a]	8.7	138.1[a]	8.9

[a]: One animal was excluded from evaluation due to not pregnant

One generation reproduction study. P-generation; Bodyweight gain (g) in females (lactation)

Dose level (ppm)	**Lactation period (days)**							
	0-4		**0-7**		**0-14**		**0-21**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	17.0[a]	7.4	23.1[a]	11.5	32.7[a]	13.2	23.7[a]	15.9
2500	15.2	9.7	16.5	7.8	29.5	6.2	15.4	14.2
5000	10.9	8.7	15.4	13.2	18.8** (43%)	6.4	20.7	11.5
10000	6.6[a]	13.1	3.6**[a] (84%)	14.5	-8.6**[a]	10.8	-4.3**[a]	17.4

[a:] One animals was excluded from evaluation due to not pregnant
**: Significantly different from control group at P<0.01

One generation reproduction study. Oestrous cycle data of P generation

Level (ppm)	**No. of animals examined**	**No. of animals with normal oestrous cycle[A]**	**No. of animals with abnormal oestrous cycle**			
			Total no.	**Persistent oestrous[B]**	**Prolongation[C]**	**Others[D]**
0	10	10	0	0	0	0
2500	10	10	0	0	0	0
5000	10	9	1	0	1	0
10000	10	9	1	0	1	0

[A] Oestrous cycles showed regularly at 4 or 5 days interval
[B] Oestrous showed persistently for over 3 days
[C] Oestrous cycles showed at 6 days or longer interval
[D] Oestrous cycles showed abnormalities other than B and C

One generation reproduction study. P-generation; Reproductive findings (mean ±SD)

Reproductive performance	**0 ppm**	**2500 ppm**	**5000 ppm**	**10000 ppm**
No. of pairs	10	10	10	10
Mating index[A][%]	90	100	100	100
No. of days for copulation	3.9±3.9	2.6±1.0	2.5±1.0	2.3±0.9
No. of pregnant female	9	10	10	9
Fertility index[B][%]	100	100	100	90
Delivery index[C][%]	100	100	100	100
Parturition data				
No. of dams	9	10	10	9
Gestation period	22.2±0.4	22.2±0.4	22.1±0.3	22.1±0.3
No. of implantations	13.1±3.3	14.1±2.4	14.2±1.1	13.8±1.2
Live birth index[D][%]	94.4±5.6	91.6±4.0	97.3±3.5	94.6±5.7
Still born index[E][%]	0.0±0.0	0.0±0.0	0.7±2.2	0.0±0.0
No of delivered/litter	12.3±3.2	12.9±2.2	13.9±1.1	13.0±1.1
No. of live offspring/litter	12.3±3.2	12.9±2.2	13.8±1.1	13.0±1.1

Male	5.9±1.6	7.0±1.4	6.2±2.3	7.0±1.7
Female	6.4±2.8	5.9±2.1	7.6±2.6	6.0±1.3
No. of dead offspring/litter	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0
Sex ratio[F]	49.6±13.2	55.2±10.7	45.4±17.8	53.3±10.6

[A] (No. of females copulated/no. of females paired) x 100
[B] (No. of pregnancies/no. of females coupled) x 100
[C] (No. of dams/no. of pregnancies) x 100
[D] (No. of live offsprings/no. of implantation site) x 100
[E] (No. of dead offsprings/no. of delivered) x 100
[F] (No. of live male offsprings/no. of live offsprings) x 100

One generation reproduction study. P-generation; Cholinesterase activity [IU/g] in the brain

Dose level (ppm)	**Cholinesterase activity in the brain**			
	Male		**Female**	
	Mean±SD [IU/g]	**Relative activity to control values as 100% [%]**	**Mean ±SD [IU/g]**	**Relative activity to control values as 100% [%]**
0	11.62±0.31	100.0	10.53±0.70	100.0
2500	11.32±0.18	97.4	9.95±0.54	94.5
5000	10.98**±0.49 (6%)	94.5	9.40**±0.40 (11%)	89.3
10000	10.30**±0.35 (11%)	88.6	8.60**±0.56 (18%)	81.7

Control group and 10000 ppm group: in each group on female animal was not pregnant and hence excluded; n=9
**Significantly different from the control group; p<0.01

One generation reproduction study. P-generation; Absolute organ weight (g) in males and females

Dose level (ppm)	**Liver**		**Testes/ovaries**		**Epididymides/uterus**	
Males	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	16.5282	1.7246	3.4876	0.3891	1.2116	0.1083
2500	16.9876	1.0419	3.2873	0.3046	1.1376	0.1238
5000	17.5913	1.6049	3.4793	0.2131	1.1817	0.0981
10000	19.2905** (17%)	1.8433	3.6281	0.5093	1.1163	0.1369
Females						
0	14.2980	0.7977	0.0901	0.0083	0.4006	0.0587
2500	15.5104* (8%)	0.7899	0.0846	0.0124	0.3988	0.0952
5000	17.2994** (21%)	1.7766	0.0826	0.0150	0.3424	0.0556
10000	17.9198** (25%)	1.0328	0.0591** (34%)	0.0103	0.2094** (48%)	0.0164

Control group and 10000 ppm group: in each group one female animal was not pregnant and hence excluded; n=9
**Significantly different from the control group: p<0.01

One generation reproduction study. P-generation; Organ to body weight ratio in males and females

Dose level (ppm)	**Brain**		**Liver**		**Kidneys**		**Testes/ovaries**		**Epididymides/uterus**	
Males	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	0.4502	0.0281	3.6125	0.2014	0.6945	0.0456	0.7640	0.0729	0.2663	0.0273
2500	0.4423	0.0258	3.7220	0.2054	0.7052	0.0601	0.7223	0.0847	0.2498	0.0314
5000	0.4533[1]	0.0206	3.8619	0.3029	0.7117	0.0553	0.7656	0.0625	0.2602	0.0277
10000	0.4577	0.0246	4.3569** (21%)	0.2501	0.7588* (9%)	0.05660	0.8203	0.1021	0.2527	0.0314
Females										
0	0.5950 [2]	0.0235	4.5669	0.3136	0.6401	0.0333	0.0289	0.0028	0.1309	0.0219
2500	0.6103[2]	0.0336	5.0594	0.2662	0.6604	0.0443	0.0271	0.0036	0.1324	0.0370
5000	0.6291[2]	0.0425	5.8223** (27%)	0.6095	0.7217** (13%)	0.0437	0.0274	0.0050	0.1179	0.0175
10000	0.6907** (16%)	0.0419	6.8902** (51%)	0.3892	0.7584** (18%)	0.0386	0.0229* (21%)	0.0043	0.0804** (39%)	0.0070

Control group and 10000 ppm group: in each group one female animal was not pregnant and hence excluded; n=9
[1] One organ was omitted from the statistical analysis due to human error
[2] One final body weight was lost by human error at sacrifice
*Significantly different from the control group; p<0.05
**Significantly different from the control group; p<0.01

One generation reproduction study. F1 generation; Summary of body weight (g) of offspring during lactation period

Dose level (ppm)	**Post-natal day**									
	0		**4**		**7**		**14**		**21**	
Male	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	7.07	0.96	11.71	2.21	19.18	2.73	37.46	3.51	64.07	5.46
2500	6.77	0.62	11.22	1.23	18.65	1.43	35.60	2.83	60.73	3.77
5000	6.22	0.43	10.14	1.02	16.96* (12%)	1.35	32.57* (13%)	1.73	54.34** (15%)	3.37
10000	6.03** (15%)	0.35	9.42* (20%)	0.75	14.64* (24%)	0.71	23.87** (36%)	1.20	34.79** (46%)	2.75
Female										
0	6.79	0.85	11.21	1.84	18.03	2.30	35.94	2.68	61.26	4.61
2500	6.25	0.54	10.71	1.09	18.18	1.60	35.56	2.15	58.36	3.54
5000	5.97	0.53	9.63* (14%)	0.85	15.96* (11%)	1.38	31.00** (14%)	1.54	51.07** (17%)	3.12
10000	5.66** (17%)	0.27	8.90** (21%)	0.82	13.91** (23%)	1.15	23.06** (36%)	1.36	34.21** (44%)	1.74

Control group and 10000 ppm group: no. of litter=9; 2500 and 5000 ppm groups: no. of litter= 10
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of body weights (g) of offspring after weaning

Dose level (ppm)	**Post-natal day**											
	21		**28**		**35**		**42**		**49**		**56**	
Male	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	65.1	4.9	109.1	7.5	170.3	11.1	231.1	15.8	295.8	19.2	357.6	18.3
2500	60.0* (8%)	5.2	98.8* (9%)	10.4	155.2	16.1	217.2	17.8	278.2	18.4	336.9	23.2
5000	55.0** (16%)	3.2	91.8** (10%)	3.8	146.5** (14%)	4.6	206.1** (11%)	8.7	265.5** (10%)	10.3	321.9** (10%)	11.0
10000	34.2** (47%)	3.2	61.2** (44%)	6.9	102.8** (40%)	11.6	146.2** (37%)	16.0	194.6** (34%)	20.0	246.7** (31%)	24.3
Female												
0	61.2	4.2	98.4	7.0	143.2	8.1	176.8	9.1	203.1	10.5	227.3	12.0
2500	58.8	3.5	90.7* (8%)	5.1	131.8* (8%)	9.2	165.0* (7%)	11.3	180.6	29.2	211.3* (7%)	12.6
5000	51.2** (16%)	3.5	81.2** (17%)	6.2	119.6** (16%)	9.6	155.1** (12%)	12.3	181.6* (11%)	14.6	201.3** (11%)	15.1
10000	34.2**[A] (44%)	1.8	57.6** (41%)	3.9	90.5** (37%)	5.7	122.3** (31%)	7.6	148.8** (27%)	11.0	172.1** (24%)	10.8

[A] One body weight was lost by human error
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of bodyweight gain (g) of offspring during lactation

Dose level (ppm)	**Post-natal day**							
	0-4		**0-7**		**0-14**		**0-21**	
Males	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	4.64	1.34	12.14	1.95	30.42	2.89	57.04	4.86
2500	4.45	0.71	11.82	1.06	29.56	1.85	53.90	3.54
5000	3.92	0.68	10.72* (12%)	1.02	26.33** (13%)	1.53	48.10** (16%)	3.06
10000	3.38* (27%)	0.57	8.54* (30%)	0.73	17.77** (42%)	1.37	28.69** (50%)	2.83
Females								
0	4.42	1.12	11.29	1.58	29.21	1.96	54.52	3.85
2500	4.46	0.65	11.87	1.07	29.26	1.81	52.06	3.18
5000	3.66	0.42	10.03	1.00	25.08** (14%)	1.31	45.15** (17%)	2.81
10000	3.24* (27%)	0.65	8.22** (27%)	0.99	17.36** (41%)	1.47	28.52** (48%)	1.86

Control group and 10000 ppm group: no. of litter=9; 2500 and 5000 ppm groups: no. of litter= 10
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of bodyweight gain (g) of offspring after weaning

Dose level (ppm)	**Post-natal day**									
	21-28		**21-35**		**21-42**		**21-49**		**21-56**	
Males	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	44.0	3.4	105.2	7.5	166.0	12.5	230.7	16.1	292.5	15.5
2500	38.8	6.7	95.2	12.7	157.2	15.0	218.2	16.3	276.9	22.2
5000	36.8** (16%)	2.1	91.5** (13%)	4.2	151.1* (9%)	8.1	210.5* (9%)	9.6	266.9** (9%)	10.7
10000	27.0** (39%)	4.1	68.6** (35%)	8.9	112.0** (33%)	13.7	160.4** (30%)	18.1	212.5** (27%)	22.3
Females										
0	37.2	4.4	82.0	7.0	115.6	9.8	141.9	10.9	166.1	13.5
2500	31.9** (14%)	2.5	73.0* (11%)	6.4	106.2	8.6	121.8* (14%)	28.5	152.5	10.5
5000	30.0** (19%)	4.3	68.4** (17%)	8.6	103.9* (10%)	11.9	130.4	14.5	150.1* (10%)	15.1
10000[A]	23.0** (38%)	3.6	55.7** (32%)	5.3	88.1** (24%)	7.7	115.2** (19%)	11.7	138.9** (16%)	11.2

[A] One body weight was lost by human error at post-natal day 21
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Anogenital distance data of offspring (mm, Mean±S.D.)

Dose level (ppm)	**AGD**	**AGD/3√BW**
Male		
0	3.162±0.345 (n=9)[a]	1.64.742±13.065 (n=9)
2500	3.150±0.323 (n=10)	166.259±13.314 (n=10)
5000	3.057±0.472 (n=10)	165.862±22.812 (n=10)
10000	2.869±0.220 (n=9)	157.547±12.120 (n=9)
Female		
0	1.224±0.103 (n=9)	64.668±3.803 (n=9)
2500	1.245±0.088 (n=10)	67.587±4.643 (n=10)
5000	1.197±0.100 (n=10)	66.022±5.456 (n=10)
10000	1.162±0.076 (n=9)	65.214±4.775 (n=9)

[a]: n=No. of Litter
AGD: Anogenital distance

One generation reproduction study. F1 generation; Vaginal opening data of offspring (mean±S.D.)

Dose level (ppm)	Vaginal opening complete day	B.W. at complete day (g)
0	32.6±2.2 (n=10)	128.0±15.7
2500	32.7±1.8 (n=10)	118.6±10.7
5000	33.1±1.9 (n=10)	108.5±12.0**
10000	33.7±3.7 (n=10)	83.5±12.7**

n= number of animals
**Significantly different from control group at P<0.01

One generation reproduction study. F1 generation; Summary of preputial separation in males

Dose level (ppm)	Preputial separation				Body weight at complete day (g)	
	Initial day		Complete day			
Male	Mean	SD	Mean	SD	Mean	SD
0	40.8	1.5	43.6	1.2	248.2	17.0
2500	41.0	2.2	44.3	1.3	237.2	14.4
5000	41.3	1.2	43.8	1.5	224.0**	10.6
10000	43.8**	2.2	46.0**	2.2	174.0**	14.3

**Significantly different from control group at P<0.01

One generation reproduction study. F1 generation: Summary of absolute organ weights (g) at PND 21

Dose level (ppm)	**No. of animals examined**	**Brain**		**Thymus**		**Spleen**		**Uterus**	
Male		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	9	1.5208	0.0811	0.2790	0.0552	0.3394	0.0646	-	
2500	10	1.5032	0.0655	0.2459	0.0151	0.2669** (21%)	0.586	-	
5000	9	1.4300* (6%)	0.0449	0.2184	0.0298	0.2234** (34%)	0.0388	-	-
10000	9	1.3169** (13%)	0.0618	0.1288** (54%)	0.0293	0.1106** (67%)	0.0250	-	
Female									
0	9	1.4481	0.0935	0.2589	0.0425	0.3063	0.0392	0.0339	0.0059
2500	10	1.4463	0.0297	0.2442	0.0319	0.2519* (18%)	0.0445	0.0324	0.0028
5000	10	1.4026	0.0478	0.2300	0.0168	0.2148** (30%)	0.0501	0.0298	0.0042
10000	9	1.1434** (21%)	0.3826	0.1379** (47%)	0.0310	0.1141** (63%)	0.0229	0.0221** (35%)	0.0033

PND: post-natal day
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of organ to body weight ratio (g/100 g bw) at PND 21

Dose level (ppm)	**No. of animals examined**	**Brain**		**Thymus**		**Spleen**		**Uterus**	
Male		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	9	2.4139	0.3047	0.4382	0.0741	0.5314	0.744	-	
2500	10	2.4736	0.1077	0.4054	0.0369	0.4370* (18%)	0.0811	-	
5000	9	2.7039* (12%)	0.1621	0.4132	0.0613	0.4220** (21%)	0.0745	-	-
10000	9	3.7642** (56%)	0.2309	0.3642* (17%)	0.0675	0.3133** (41%)	0.0583	-	

Female									
0	9	2.4152	0.2304	0.4294	0.0586	0.5088	0.0525	0.0561	0.0086
2500	10	2.5142	0.1594	0.4221	0.0394	0.4353	0.0656	0.0562	0.0059
5000	10	2.7348** (13%)	0.1087	0.4491	0.0441	0.4185* (18%)	0.0996	0.0579	0.0066
10000	9	3.7062** (53%)	0.2529	0.3977	0.0784	0.3284** (35%)	0.0509	0.0640	0.0074

PND: post-natal day
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of absolute organ weights (g) at PND 56

Dose level (ppm)	**No. of animals examined**	**Brain**		**Kidneys**		**Testes/ovaries**		**Epididymides/uterus**		**Seminal vesicles**		**Prostate**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Male													
0	10	2.0404	0.0896	2.7263	0.1825	2.9795	0.1664	0.5267	0.0350	0.7245	0.0990	0.6500	0.0970
2500	10	1.9805	0.0685	2.7781	0.3558	3.0045	0.1598	0.5075	0.0513	0.6914	0.0976	0.6718	0.1053
5000	10	1.9300** (5%)	0.0707	2.8920	0.1958	3.0837	0.2593	0.5274	0.0522	0.7367	0.0978	0.6933	0.0545
10000	10	1.7188** (16%)	0.0832	2.3179** (15%)	0.2762	2.7258	0.3154	0.4175** (21%)	0.0498	0.5261** (27%)	0.1299	0.5675	0.1456
Female													
0	10	1.8618	0.0783	1.7426	0.1721	0.0811	0.0172	0.3699	0.0753	-	-	-	-
2500	10	1.8158	0.0890	1.8689	0.2116	0.0710	0.0148	0.3678	0.1125	-	-	-	-
5000	10	1.7502** (6%)	0.0564	1.8507	0.2234	0.0705	0.0131	0.3213	0.0698	-	-	-	-
10000	10	1.6362** (12%)	0.0795	1.5912	0.1579	0.0641* (21%)	0.0078	0.3605	0.1331	-	-	-	-

PND: post-natal day
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of organ to body weight ratio (g/100 g bw) at PND 56

Dose level (ppm)	**No. of animals examined**	**Brain**		**Liver**		**Kidneys**		**Testes/ovaries**	
Male		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	10	0.5712	0.0252	4.3101	0.2096	0.7634	0.0533	0.8340	0.0419
2500	10	0.5901	0.0394	4.6953** (9%)	0.2910	0.8240	0.0804	0.8944	0.0604
5000	10	0.6001	0.0279	5.0150** (16%)	0.2770	0.8984** (18%)	0.0500	0.9589** (15%)	0.0868
10000	10	0.7000** (23%)	0.0419	5.9989** (39%)	0.2529	0.9385** (23%)	0.0435	1.1082** (33%)	0.1151

Female									
0	7[A]	0.8089	0.0538	4.0241	0.3428	0.7571	0.0531	0.0319	0.0051
2500	8[B]	0.8653	0.0602	4.4259	0.2401	0.8981** (19%)	0.0769	0.0326	0.0058
5000	10	0.8672	0.0515	5.0042** (24%)	0.3140	0.9134** (21%)	0.0855	0.0347	0.0060
10000	10	0.9535** (18%)	0.0651	5.8169** (45%)	0.3824	0.9242** (22%)	0.0607	0.0374	0.0042

PND: post-natal day
[A] Three final body weights were lost by human error at sacrifice
[B] Two final body weights were lost by human error at sacrifice
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Summary of organ to body weight ratio (g/100 g bw) at PND 56 (continued)

Dose level (ppm)	**No. of animals examined**	**Epididymides/uterus**		**Prostate**	
Male		**Mean**	**SD**	**Mean**	**SD**
0	10	0.1476	0.0128	0.1823	0.0292
2500	10	0.1509	0.0161	0.2003	0.0348
5000	10	0.1638	0.0157	0.2157	0.0198
10000	10	0.1696** (15%)	0.0148	0.2278** (25%)	0.0417
Female					
0	7[A]	0.1714	0.0450	-	-
2500	8[B]	0.1849	0.0682	-	-
5000	10	0.1588	0.0329	-	--
10000	9	0.2083	0.0723	-	

PND: post-natal day
[A] Three final body weights were lost by human error at sacrifice
[B] Two final body weights were lost by human error at sacrifice
*Significantly different from control group; p<0.05
**Significantly different from control group; p<0.01

One generation reproduction study. F1 generation; Differential ovarian follicle counts data of offspring at PND 56 (mean±S.D.)

Dose level (ppm)	**No. of follicles**		
	Primordial	**Growing**	**Antral**
0	211.1±101.6 (n=10)	89.0±31.9 (n=10)	59.3±20.1 (n=10)
2500	180.9±65.8 (n=10)	79.7±15.2 (n=10)	51.2±5.0 (n=10)
5000	130.7±51.5 (n=10)	73.5±15.7 (n=10)	50.1±13.5 (n=10)
10000	200.6±93.6 (n=10)	76.0±22.2 (n=10)	64.1±18.4 (n=10)

10.10.3 Comparison with the CLP criteria

According to CLP Guidance Annex 1: 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…"

Three generation reproductive toxicity study:

Administration of tolclofos-methyl at dietary concentrations of up to 1000 ppm (70.6 mg/kg bw/day) did not have any effect on mating performance or fertility, and no treatment-related effects were noted in the offspring. Thus, no concern for classification was observed in this study.

One generation reproductive toxicity study:

Administration of tolclofos-methyl at dietary concentrations of up to 10000 ppm (668 mg/kg bw/day) did not have any effect on mating performance or fertility. Parental adverse findings were noted at ≥5000 ppm (≥338 mg/kg bw/day). The parental findings included reduced bodyweight and bodyweight gain and effects on the liver and kidney (increased organ weights). For the offspring generation reduced body weights and bodyweight gains were noted at ≥2500 ppm (≥173 mg/kg bw/day). At 2500 ppm reduced pup weight (F1 generation: M: 8%) were noted on PND 21 but no statistically significant reductions were noted during lactation. The effect seems to be a consequent of higher intake in pups than parents and not due to higher sensitivity. A delay of starting and completing separation was noted in the offspring at 10000 ppm but this finding was considered a secondary effect due to body weight suppression. Effects on the liver and kidney (increased organ weights) were noted in the offspring generation at ≥5000 ppm (519 mg/kg bw/day), and increased relative prostate weight was noted in addition at 10000 ppm (1161 mg/kg bw/day). The observerd effects were not considered of concern for a classification.

10.10.4 Adverse effects on development

Table 43: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Developmental toxicity Oral (gavage) In house method Rat Fischer 344 CD®F F 30/dose GLP: No	Tolclofos-methyl (purity: 94.9%) Vehicle: 0.5% methylcellulose 0, 5, 15, 50 mg/kg bw/day Gestation Days 6-15	Maternal effects: 5 and 15 mg/kg bw/day: No treatment-related effects 50 mg/kg bw/day: ↓ mean implantation efficiency (86.1% compared to 91.9% in control group) Developmental effects: 5, 15 and 50 mg/kg bw/day: No treatment-related effects NOAEL maternal and developmental: ≥50 mg/kg bw/day	RAR Vol.3 B.6.6.2.1/01
Developmental toxicity Oral (gavage) EPA 83-3 Rat Sprague Dawley F 23/dose GLP: No	Tolclofos-methyl (purity: 96.7%) Vehicle: 0.5% methylcellulose 0, 100, 300 and 1000 mg/kg bw/day Gestation Days 6-15	Maternal effects: 100 mg/kg bw/day: No treatment-related effects 300 mg/kg bw/day: No treatment-related effects 1000 mg/kg bw/day: ↓bw gain during Days 6-11 (27%) (net bodyweight change of 14% showed a statistically significant negative trend) Developmental effects: 100 mg/kg bw/day: No treatment-related effects 300 mg/kg bw/day: No treatment-related effects 1000 mg/kg bw/day: - increased incidence of unossified 5th and 6th sternebrae NOAEL maternal and developmental: 300 mg/kg bw/day	RAR Vol. 3 B.6.6.2.1/02

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity Oral (gavage) In house method Rabbit New Zealand White F 13-17/dose GLP: No *Few animals were used in study (OECD TG 414 recommends sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy)*	Tolclofos-methyl (purity: 98.7%) 0, 300, 1000, 3000 mg/kg bw/day Vehicle: 0.5% carboxymethylcellulose Gestation Days 6-18	<u>Maternal effects:</u> <u>300 mg/kg bw/day</u> ↓bw (Day 29: 10%) <u>1000 mg/kg bw/day:</u> -abortion (one dam on GD 26) ↓bw (Day 29: 11%) ↓bw gain (56%) ↓FC -organ weight changes (↓kidney 12%) <u>3000 mg/kg bw/day:</u> -mortality (one dam died on GD 14) -abortions (two dams on GD 20 and 22, respectively) ↓bw (Day 29:11%) ↓bw gain (76%) ↓FC -organ weight changes (↓kidney 13%, ↓spleen 20%) <u>Developmental effects:</u> <u>300 mg/kg bw/day:</u> No treatment-related effects <u>1000 and 3000 mg/kg bw/day:</u> abortions NOAEL maternal and developmental: 300 mg/kg bw/day	RAR Vol. 3 B.6.6.2.1/03

F: female

Table 44: Summary table of human data on adverse effects on development

No data

Table 45: 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

Two teratology studies in rats were performed. In the first study the highest applied dose was 50 mg/kg bw/day. No adverse maternal toxicity or embryo-toxic, or teratogenic effects were noted in the study. NOAEL was set at ≥50 mg/kg bw/day. The dose levels used in the study were low (B.6.6.2.1/01).

Rat study (B.6.6.2.1/01 in RAR). Mean maternal body weights (g) and bodyweight changes (%)

Body weight	Day	Dose level group (mg/kg bw/day)			
		0	5	15	50
Sample size (N):a		21	23	26	26
Mean weight (g)	0	149.6	148.9	148.3	150.3
	6	166.0	165.2	164.8	165.9
	11	181.8	181.5	179.2	181.0
	15	196.5	195.4	194.2	195.3
	19	224.9	223.3	221.2	224.3
Mean change (g)	0-6	16.4	16.3	16.6	15.7
	6-15	30.4	30.2	29.3	29.3
	15-19	28.4	28.0	27.0	29.0
	0-19	75.2	74.4	72.9	74.0
Percent change (%)	0-6	11.0	10.9	11.2	10.4
	6-15	18.3	18.3	17.8	17.7
	15-19	14.5	14.3	13.9	14.8
	0-19	50.3	50.0	49.2	49.2

[a] Only data from pregnant rats were included in calculations of the mean values

Rat study (B.6.6.2.1/01 in RAR). Mean maternal food consumption values [a]

Day	Dose level group (mg/kg bw/day)			
	0	5	15	50
	21	23	26	26
Pretreatment period (Days 0-6)	66.2	62.4	60.7	61.5
Treatment period (Days 6-11)	62.6	62.4	61.3	61.6
Treatment period (Days 11-15)	52.2	55.1	53.9	55.3
Posttreatment period (Days 15-19)	57.1	55.5	59.1	58.5
Total food consumption (Days 0-19)	238.1	235.4	234.9	237.0

Rat study (B.6.6.2.1/01 in RAR). Summary of the ovarian, uterine, and litter data

Dose level	0 mg/kg bw/day	5 mg/kg bw/day	15 mg/kg bw/day	50 mg/kg bw/day
Number of females mated	30	30	30	30
Number of rats pregnant	21	23	26	26
Pregnancy rate (%)	70.0	76.7	86.7	86.7
Mean number of:				

Corpora lutea	11.0	11.7	11.3	11.2
Implantations	10.1	9.6	9.6	9.6
Resorptions	1.0	0.7	0.8	0.4
Fetuses-dead	0	0.1	0	0.04
Fetuses-alive	9.1	8.9	8.8	9.2
Indices calculated on per litter basis:				
Mean implantation efficiency (%)	91.9	83.3	85.5	86.1*
Mean incidence of resorption (%)	10.0	7.0	9.2	4.4
Mean incidence of foetal death (%)	0	1.0	0	0.4
Mean incidence of foetal viability (%)	90.0	92.1	90.8	95.2
Live male foetuses				
Mean body weight (g)	2.21	2.21	2.20	2.22
Mean crown-rump distance (cm)	3.21	3.18	3.20	3.18
Live female foetuses				
Mean body weight (g)	2.11	2.10	2.11	2.14
Mean crown-rump distance (cm)	3.15	3.11	3.15	3.12
Mean sex ratio	1.17	1.15	1.83	1.40
Mean uterine weight (g)	36.13	34.99	34.87	37.38

*Significantly different from control ($p \leq 0.05$)

Rat study (B.6.6.2.1/01 in RAR). Foetal visceral and skeletal variations

Dose level	0 mg/kg bw/day	5 mg/kg bw/day	15 mg/kg bw/day	50 mg/kg bw/day
Total number of litters examineda	21	23	26	26
Mean number of				
Visceral anomalies	0	0	0	0
Visceral variants	0	0	0	0.04
Skeletal anomalies	0	0	0	0
Skeletal variants	0.5	1.1	0.9	0.8
Indices calculated on per litter basis				
Mean incidence of visceral anomalies (%)	0	0	0	0
Mean incidence of visceral variants (%)	0	0	0	1.3
Mean incidence of skeletal anomalies (%)	0	0	0	0
Mean incidence of skeletal variants (%)	7.5	17.3	13.1	12.2

[a:] Only data from live pups were used in calculations of means

In the second study dose levels up to 1000 mg/kg bw/day were tested. Treatment was associated with reduced maternal bodyweight gain (Days 6-11: 27%, net bodyweight change of 14% showed a statistically significant negative trend) noted in dams at 1000 mg/kg bw/day. Delayed ossification of the 5th and/or 6th sterneabrae was seen in the fetuses at 1000 mg/kg bw/day. The NOAEL for maternal toxicity was set at 300 mg/kg bw/day. NOAEL for developmental toxicity was set at 300 mg/kg bw/day based on increased incidence of non-ossified sternebrae noted at 1000 mg/kg bw/day (B.6.6.2.1/02).

Comment: Maternal NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Experts´consultation 2.7). The maternal and developmental NOAEL was concluded to be set at 300 mg/kg bw/day.

Rat study (B.6.6.2.1/02 in RAR). Mean maternal body weights (g), bodyweight gains (g) and food consumption (g) during gestation

Dose level	0 mg/kg bw/day	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Body weight				
Day 20	366	360	363	362
Gravid Uterine Weight	79.9	76.7	78.6	80.9
Carcass Weight	286.3	282.7	284.3	281.2
Bodyweight gain				
Day 0-6	34	35	34	32
Day 6-7	0	0	-2	-1
Day 6-11	18	16*	16	13* (27%)
Day 6-20	107	103	105	102
Day 16-20	57	58	56	57
Day 0-20	141	137	138	134
Net Body Weight Changea	61.6	60.4	59.2	52.9
Food consumption				
Day 6-16a	239	238	234	229
Day 16-20a	102	104	101	98

*Significantly different from control ($p \leq 0.05$)
a: Significant negative trend ($p \leq 0.05$)

Rat study (B.6.6.2.1/02 in RAR). Teratogenicity in rats: Caesarean data

Dose level (mg/kg bw/day)	0	100	300	1000
Number (%) of:				
Females mated	23	23	23	23
Females surviving to Day 20 cesarean section	23 (100)	23 (100)	23 (100)	23(100)
Females pregnant	19 (83)	22 (96)	23 (100)	22 (96)
Pregnant females surviving to Day 20 cesarean section	19 (100)	22 (100)	23 (100)	22 (100)
Females delivering early	0 (0)	0 (0)	0 (0)	0 (0)
Litters examined	19	22	23	22
Mean number (%) of:				
Corpora lutea	17.6a	16.7	16.9	17.1
Implantations	15.3	15.0	14.8	15.8
Live foetuses	14.8 (97)	14.2 (95)	14.3 (97)	15.0 (95)
Dead foetuses	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Early resorptions	0.5 (3)	0.7 (5)	0.5 (3)	0.8 (5)
Late resorptions	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Total resorptions	0.5 (3)	0.7 (5)	0.5 (3)	0.8 (5)
Male foetuses	7.7 (52)	7.4 (52)	7.3 (51)	7.7 (52)

Mean:				
Implantation efficiency (%)	90a	90	89	93
Foetal weight (g)	3.3	3.5	3.5	3.4
Male	3.4	3.5	3.6	3.4
Female	3.3	3.4	3.4	3.3
Covariate adjusted mean:				
Foetal weight (g)	3.3	3.5	3.5	3.4
Male	3.4	3.5	3.6	3.4
Female	3.3	3.4	3.4	3.3

Rat study (B.6.6.2.1/02 in RAR). Teratogenicity in rats: Foetal skeletal variations

Dose level (mg/kg bw/day)		0	100	300	1000
Litters evaluated	No.	19	22	23	22
Foetuses evaluated	No.	139	154	163	168
Live	No:	139	154	163	168
Dead	No.	0	0	0	0
5th sternebrae unossified					
Foetal incidence T	No.	41	46	52	75*
	%	29	30	32	45
Litter incidence	No.	14	14	13	18
	%	74	64	57	82
6th sternebrea unossified					
Foteal incidence	No.	5	12	4	19*
	%	3.6	7.8	2.5	11
Litter incidence	No.	4	6	3	8
	%	21	27	13	36

Rat study (B.6.6.2.1/02 in RAR). Teratogenicity in rats: Historical control data. Foetal skeletal variations

Dose level (mg/kg bw/day)		**0**	**100**	**300**	**1000**	**HCD 1994-1998**	
Litters evaluated	No.	19	22	23	22	317	
Foetuses evaluated	No.	139	154	163	168	2247	
Live	No:	139	154	163	168		
Dead	No.	0	0	0	0		
5th sternebrae unossified							
Foetal incidence						**Mean [%]**	**Range [%]**
	No.	41	46	52	75*		
	%	29	30	32	45	35.92	0-70

Litter incidence	No.	14	14	13	18		
	%	74	64	57	82	76.7	0-100
6th sternebrea unossified							
Foteal incidence	No.	5	12	4	19*		
	%	3.6	7.8	2.5	11	9.03	0-46
Litter incidence	No.	4	6	3	8		
	%	21	27	13	36	23.1%	0-95

The teratology study in rabbits showed maternal toxicity with mortality, abortions, and reduced body weight and bodyweight gain. Embryofetal development was not impaired. NOAEL for maternal toxicity was set at 300 mg/kg bw/day based on abortions noted at ≥1000 mg/kg bw/day, mortality noted in one dam at 3000 mg/kg bw/day, reduced body weight and bodyweight gain noted at ≥1000 mg/kg bw/day. NOAEL for developmental toxicity was set at 300 mg/kg bw/day based on abortions noted at ≥1000 mg/kg bw/day (B.6.6.2.1/03).

Comment: Maternal and developmental NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Experts´consultation 2.8). The maternal NOAEL was agreed to be set at 300 mg/kg bw/day based on reduced bodyweight gain and abortion. Abortion was also considered the critical effect for developmental toxicity and the NOAEL for developmental was set at 300 mg/kg bw/day.

Rabbit study (B.6.6.2.1/03 in RAR). Body weight and bodyweight gain

Dose level	0 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	3000 mg/kg bw/day
Body weight (kg)				
Day 0	3.00	2.71* (10%)	2.64** (12%	2.75* (8%)
Day 29	3.68	3.32* (10%)	3.26*(11%)	3.29* (11%)
Bodyweight gain (kg)				
Day 0-29	0.68	0.62	0.65	0.55
Day 6-19	0.34	0.27	0.15* (56%)	0.08** (76%)

Significantly different from control (p≤0.05)
**Significantly different from control (p≤0.01)

Rabbit study (B.6.6.2.1/03 in RAR). Food consumption

Dose level	0 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	3000 mg/kg bw/day
Day 0-6 (Average)b	151.25	136.07	144.82	142.85
Day 7	155.20	125.71	111.08**	136.12
Day 8	155.87	138.57	117.08*	120.12**
Day 9	147.87	139.43	99.54**	108.94**
Day 10	163.73	150.86	110.00***	128.35*
Day11	166.80	160.71	135.85*	131.53*
Day 12	155.60	137.00	123.08**	111.88**
Day 13	149.33	136.71	136.15	111.29*
Day 14	144.80	132.57	128.15	114.50*

Day 16	139.47	116.14	125.54	124.88
Day 17	142.40	123.43	118.62	125.75
Day 18	141.60	134.29	120.00	125.75
Day 19	138.27	120.43	112.08	125.00
Day 28-29 (Total of 2 days)c	254.00	231.86	220.83	251.71
Day 28-29 (Average)b	127.00	115.93	110.42	125.86

[a]: g/day
[b]: g/day
[c]: g/2 days
*Significantly different from control (p≤0.05)
**Significantly different from control (p≤0.001)

Rabbit study (B.6.6.2.1/03 in RAR). Organ weights of female rabbits treated orally with tolclofos-methyl

Dose level	0 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	3000 mg/kg bw/day
Body weight (kg)	3.68	3.32* (10%)	3.26* (11%)	3.29* (11%)
Kidney (right) (g)	9.19	8.60	8.50	8.20* (11%)
Kidney (left) (G)	9.35	8.61	8.22* (12%)	8.18* (13%)
Spleen (g)	1.47	1.52	1.76	1.17* (20%)
Liver (g)	104.54	92.05* (12%)	94.87	106.91
Urinary bladder (g)	1.84	1.47* (20%)	1.45* (21%)	1.63 (11%)

*Significantly different from control (p<0.05)
N=15

Rabbit study (B.6.6.2.1/03 in RAR). Caesarean data

Dose level	0 mg/kg bw/day	3mg/kg bw/day	1000 mg/kg bw/day	3000 mg/kg bw/day
Number of animals	15	14	12	14
No. of implantations				
Total	124	121	102	109
Mean	8.27	8.64	8.50	7.79
No. of lived foetuses				
Total (%)	113 (91.1%)	109 (90.1)	94 (92.2)	89 (81.7)
Mean	7.53	7.79	7.83	6.36
No. of dead foetuses				
Total	0	0	0	0
%	0	0	0	0
Resorbed embryo				
Total (%)	11 (8.9)	12 (9.9)	8 (7.8)	20 (18.3)
Implantation trace				
Total (%)	0 (0)	0 (0)	0 (0)	8 (7.3%)
Placental remnant				
Total	3 (2.4)	4 (3.3)	3 (2.9)	4 (3.7)

Early resorption				
Total	3 (2.4)	3 (2.5)	3 (2.9)	5 (4.6)
Late resorption				
Total (%)	4 (3.2)	4 (3.3)	1 (1.0)	2 (1.8)
Macerate foetus				
Total (%)	1 (0.8)	1 (0.8)	1 (1.0)	1 (0.9)
Immature infant				
Total (%)	10 (8.8)	27 (24.8)	7 (7.4)	13 (14.6)
Malformation				
Total (%)	0 (0)	0 (0)	0 (0)	0 (0)
Foetus body weight (g)				
Male				
Number (of litters)	13	14	11	12
Mean	42.38	37.27	38.93	38.62
Female				
Number (of litters)	15	13	12	13
Mean	38.14	34.45	36.40	36.26
Sex ratio				
Male				
Number (of litters)	13	14	11	12
Total (%)	58 (51.3)	64 (58.7)	45 (4.09)	46 (51.7)
Mean	4.46	4.57	4.09	3.83
Female				
Number (of litters)	15	13	12	13
Total (%)	55 (48.7)	45 (41.3)	49 (52.1)	43 (48.3)
Mean	3.67	3.46	4.08	3.31

10.10.6 Comparison with the CLP criteria

According to Regulation 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on evidence 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 1B 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 with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other 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 substance in Category 1"*

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

Rat developmental toxicity study (B.6.6.2.1/01)

Reduced mean implantation efficiency (86.1% compared to 91.9% in control group) were noted at 50 mg/kg bw/day. In the absence of other effects, this effect was not considered adverse. Thus no concern for classification was observed in this study.

Rat developmental toxicity study (B.6.6.2.1/02)

Maternal findings were noted at 1000 mg/kg bw/day and consisted of reduced bodyweight gain (27%) and reduced net bodyweight change of 14% (statistically significant negative trend). Delayed ossification of sterneabrae was seen in the fetuses at 1000 mg/kg bw/day. The incidence of delayed ossification of sterneabrae occurred at the high dose level only in the presence of maternal toxicity. Thus, no classification with regard to developmental toxicity is proposed.

Rabbit developmental toxicity study (B.6.6.2.1/03)

Adverse maternal findings were noted at ≥1000 mg/kg bw/day. These findings consisted of mortality (one dam at 3000 mg/kg bw/day), abortions (noted at ≥1000 mg/kg bw/day), reduced bodyweight and bodyweight gain (noted at ≥1000 mg/kg bw/day). The effect on abortion observed in rabbit developmental study was considered suitable for the setting of adversity but not enough for classification as the incidence of abortion was very low and observed at the limit dose of the OECD guideline (1000 mg/kg bw per day).

10.10.7 Adverse effects on or via lactation

Table 46: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Three-generation Oral (dietary) In house method Rat Sprague Dawley CD albino M, F P1: 30/sex/group P2: 100 /sex/generation P3: 25/sex/group No GLP but a QA statement *Primordial follicles and sperm parameters, and sexual maturation were not investigated in study. prostate, seminal vesicles, pituitary, thyroid and uterus were not weighed. Histopathological examination was not performed for vaigna and epididymides. Dose levels were low.*	Tolclofos-methyl (purity: 98.7%) 0, 100, 300, 1000 ppm. Equivalent to (during 15 weeks pre-mating): P1: 0, 6.9, 20.5, 70.6 mg/kg bw/day in males; 0, 8.9, 26.2, 90.5 mg/kg bw/day in females P2: 0, 7.9, 23.4, 79.6 mg/kg bw/day in males; 0, 9.2, 26.9, 98.5 mg/kg bw/day in females P3: 0, 7.6, 23.8, 78.2 mg/kg bw/day in males; 0, 9.0, 28.4, 96.1 mg/kg bw/day in females 15 weeks prior to mating and during mating, gestation and lactation.	<u>100 ppm:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is obscure, since the values fell within the normal range of the laboratory) <u>300 ppm:</u> <u>Parental:</u> ↓bw in P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is obscure, since the values fell within the normal range of the laboratory) <u>1000 ppm:</u> <u>Parental:</u> ↓bw in P2 and P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is obscure, since the values fell within the normal range of the laboratory) NOAEL: Parental and pups: ≥1000 ppm (≥70.6 mg/kg bw/day) NOAEL: Reproductive: ≥1000 ppm (≥70.6 mg/kg bw/day)	RAR Vol. 3 B.6.6.1/01
One-generation Oral (dietary)	Tolclofos-methyl (purity: 97.1%) 0, 2500, 5000, 10000 ppm. Equivalent to average intakes in the parental generation	Parental <u>2500 ppm:</u>	RAR Vol. 3 B.6.6.1/02

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
In house method Rat Crj:CD(SD)(SPF) M, F P: 10/sex/group F1: 10/sex/group GLP: No *Low number of animals in each group. Males treated 2 weeks prior to mating (OECD TG 415 recommends ten weeks prior to the mating period)*	during pre-mating in males of 0, 173, 338 and 680 mg/kg bw/day and in females of 0, 178, 353, 668 mg/kg bw/day. For the pregnant and delivered dams average intakes were 0, 175, 341 and 642 mg/kg bw/day day during the gestational period, and 0, 360, 698 and 1253 mg/kg bw/day during the lactation period. In the offspring generation average intakes were 0, 255, 519 and 1161 mg/kg bw/day in males and 0, 257, 529 and 1174 mg/kg bw/day in females. Parent females: from 2 weeks prior to mating until autopsy after weaning throughout mating, gestation and lactation periods. Parent males: from 2 weeks prior to mating to autopsy. F1 generation: from weaning to autopsy at the age of 8 weeks.	-changes in organ weights (↑ abs liver (F: 8%)) 5000 ppm: ↓bw (F: gestation period (Day 7: 5%), lactation period (5-9%)) ↓bw gain (F: lactation period 0-14 (43%)) ↓FC (F: middle to late stage of lactation period) ↓cholinesterase values in brain (M: 6%, F: 11%) -changes in organ weights (↑ abs liver (F:21%), ↑ rel liver (F:27%), ↑rel kidney (F:13%)) 10000 ppm: ↓bw (M: early premating period (Week 1: 5%); F: gestation period (5-7%), lactation period (8-20%)) ↓bw gain (M: early premating period (Weeks 0-1: 33%), F: premating period (Weeks 0-1: 51%), lactation period (period 0-7: 84%, period 0-14: -8.6 g, period 0-21: -4.3 g)) ↓FC (F: early premating, gestation, lactation) ↓cholinesterase values in brain (M: 11%, F: 18%) -organ weight changes (↑rel brain (F), ↑ abs liver (M:17%, F:25%), ↑ rel liver (M:21%, F:51%), ↑rel kidney (M:9%, F:18%), ↓abs and rel ovaries, ↓abs and rel uterus) Offsprings: 2500 ppm: ↓bw (F1 generation: M: 8% (PND 21) 9% (PND 28), F: 8% (PND 21 and 35) 7% (PND 42 and 56)) ↓bw gain (F1 generation: F: 14% (PND 21-49)) ↓FC (M, F) -organ weight changes in F1 pups (↓abs spleen (M, F), ↓rel spleen (M))	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		-organ weight changes in F1 generation (↑rel liver (M: 9%), ↑rel kidney (F: 19%)) <u>5000 ppm:</u> ↓bw (F1 pups: M: 15% (PND 21), F: 17% (PND 21); F1 generation: M: 10% (PND 56), F: 11% (PND 56)) ↓bw gain (F1 pups: M: 16% (PND 0-21); F: 17% (PND 0-21); F1 generation: M: 9% (PND 21-56), F: 10% (PND 0-21)) ↓FC (M, F) -organ weight changes in F1 pups (↓abs brain (M), ↑ rel brain (M, F), ↓abs spleen (M, F) ,↓rel spleen (M, F)) -organ weight changes in F1 generation (↓abs brain (M, F), ↑rel liver (M:16%, F: 24%), ↑rel kidney (M:18%, F:21%), ↑rel testis) <u>10000 ppm:</u> ↓bw (F1 pups: M: 46%, F: 44%; F1 generation: M: 31% (PND 56); F: 24% (PND 56)) ↓bw gain (F1 pups: M: 50% (PND 0-21); F: 48% (PND 0-21); F1 generation: M: 27% (PND 21-56), F: 16% (PND 21-56)) ↓FC (M, F) -delayed preputial separation (preputial separation completed Day 46, compared to 43.6 in controls) -organ weight changes in F1 pups (↓abs brain (M, F), ↑ rel brain (M, F), ↓abs thymus (M, F), ↓rel thymus (M), ↓abs spleen (M, F), ↓rel spleen (M, F) ↓abs uterus -organ weight changes in F1 generation (↓abs brain (M, F), ↑ rel brain (M, F), ↑rel liver (M:39%, F:45%), ↓abs kidney (M:15%), ↑rel kidney (M:23%, F:22%), ↑rel testis, ↓abs ovary, ↓abs epididymides, ↑rel	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		epididymides, ↓abs seminal vesicles, ↑rel prostate 25%) NOAEL parental: 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) NOAEL offspring generation: not established. NOAEL reproductive toxicity: ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively)	

Table 47: Summary table of human data on effects on or via lactation

No data

Table 48: 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

In the three generation reproduction study in rats neither systemic toxic effects in the parental and in the pup generations nor a reprotoxic effect was observed. The parental NOAEL was ≥1000 ppm (≥70.6 mg/kg bw/day). The NOAEL for pups and reproduction were ≥1000 ppm (≥70.6 mg/kg bw/day). The dose levels used in the study were low (RAR Vol. 3 B.6.6.1/01).

Tolclofos-methyl was further investigated in a one generation reproductive study. Systemic toxicity for both the parental and the offspring generation comprised a reduction in body weights, bodyweight gains and food consumption, and increased liver and kidney weights noted in the parental animals at ≥5000 ppm (≥338 mg/kg bw/day) and in the offspring at ≥2500 ppm (≥255 mg/kg bw/day). Furthermore, the offspring generation showed an increased prostate weight (relative weight increased 25%) at 10000 ppm (1161 mg/kg bw/day). In addition to these findings, a decrease in brain cholinesterase activity (less than 20%) was observed in the parental animals at ≥5000 ppm. The reproductive performance was not affected by tolclofos-methyl treatment. A delay of starting and completing separation was noted in the offspring at 10000 ppm. This delay was considered a secondary effect due to the body weight suppression noted at this dose level. The histopathological examination of genital organs revealed no treatment related changes. For the parental generation, the NOAEL for systemic toxicity was 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) based on reduced bodyweight gain noted in females at ≥5000 ppm and in males at 10000 ppm, reduced body weight noted in females at 10000 ppm, and organ weight changes in the liver and kidneys noted at ≥5000 ppm. NOAEL for the offspring generation could not be derived. Reduced body weights and bodyweight gains and increased organ weights of liver and kidney were noted in all treated groups. In addition, the relative weight of prostate was increased (25%) at 10000 ppm. NOAEL for reproductive toxicity was set at ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively) (RAR Vol. 3 B.6.6.1/02).

10.10.9 Comparison with the CLP criteria

According to the CLP Guidance 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 likehood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criterias (a) and (c). A reduction in pup weight was observed at Day 21 at 2500 ppm and above but not during lactation at this dose level. At higher dose levels reduced pup weights were observed in the presence of maternal toxicity. Thus the effect on bodyweight growth is 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 tolclofos-methyl

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The CLH report included a three-generation and a one-generation reproduction study in rats, as well as three developmental toxicity studies (two in rats, one in rabbits).

Adverse effects on sexual function and fertility

In the non-GLP three-generation reproduction study using an in-house method (Anon. 1985, RAR Vol.3, B.6.6.1/01), tolclofos-methyl was administered to Sprague Dawley CD albino rats (30/sex/group for P1, 25/sex/group for P2 and P3) at 0, 100, 300 or 1000 ppm in the diet for three generations. Treatment was performed during 15 weeks prior to mating and during mating, gestation and lactation. The achieved test material intakes (during 15 weeks premating) were 0, 6.9/8.9, 20.5/26.2 and 70.6/90.5 mg/kg bw/d for (m/f) P1, and 0, 7.9/9.2, 23.4/26.9 and 79.6/98.5 mg/kg bw/d for (m/f) P2, and 0, 7.6/9.0, 23.8/28.4 and 78.2/96.1 mg/kg bw/d for (m/f) P3. There were no adverse effects on reproduction, fertility and mating behaviour, and no treatment-related effects were noted in the offspring. The dose levels in this study were considered to be low, as no apparent parental toxicity was observed.

In the more recent one-generation reproduction study higher dose levels were selected. In this non-GLP study, following an in-house (OECD 415-like) method, tolclofos-methyl was administered to Crj:CD(SD)(SPF) rats (10/sex/group for P1 and F1) at 0, 2500, 5000 or 10000 ppm in the diet for one generation (Anon. 2005, RAR Vol.3 B.6.6.1/02; non-

GLP). Parent females were treated from 2 weeks prior to mating, throughout mating, gestation and lactation periods until autopsy after weaning. Parent males were treated from 2 weeks prior to mating to autopsy, and the F1 generation from weaning to autopsy at the age of 8 weeks. The achieved test material intakes were 0, 173/178, 338/353 and 680/668 mg/kg bw/d for the (m/f) parental generation during pre-mating. For the pregnant and delivering dams, this was 0, 175, 341 and 642 mg/kg bw/d during the gestational period and 0, 360, 698 and 1253 mg/kg bw/d during the lactation period. In the offspring generation (m/f) the average test material intakes were 0, 255/257, 519/529 and 1161/1174 mg/kg bw/d. There were no adverse effects on reproduction, fertility and mating behaviour, and no treatment-related histopathological findings were observed in the genital organs of the F1 generation. Parental toxicity was seen in females at 5000 and 10000 ppm and in males at 10000 ppm, and included reduced body weight and body weight gain, reduced food consumption, increased liver and kidney weights and in females also decreased uterus and ovary weights. Brain acetylcholinesterase activity was also reduced (6%/11% and 11%/18% in m/f at 5000 and 10000 ppm, respectively; activity in plasma and erythrocytes was not determined). General toxicity in the offspring was similar to that in the parental animals but started at 2500 ppm. It consisted of reduced body weight and body weight gain, reduced food consumption, increased liver and kidney weights, as well as increased prostate weight. In addition, the average day of starting and completing preputial separation was significantly later in males at 10000 ppm (start: day 43.8 versus day 40.8 in controls; completion: day 46.0 versus day 43.6 in controls). However, the DS considered this to be a secondary effect to the body weight suppression at this dose level (30% lower than controls).

Given the lack of adverse effects on mating performance and fertility, the DS concluded that tolclofos-methyl does not warrant classification for fertility.

Adverse effects on development

Rat

In a non-GLP developmental toxicity study using an in-house method (Anon. 1979, RAR Vol.3, B.6.6.2.1/01), tolclofos-methyl was administered by gavage to female Fischer 344 CD®F rats (30/dose) at 0, 5, 15 or 50 mg/kg bw/d in 0.5% methyl cellulose, on days 6-15 of gestation. Aside from a slightly reduced mean implantation efficacy in the treated groups (without a dose-response relationship), no treatment-related maternal or developmental effects were noted in this study. The highest dose level was therefore considered to be too low.

Higher doses of tolclofos-methyl were tested in a second developmental toxicity study in rats. In this non-GLP study, following an in-house (OECD 414-like) method, tolclofos-methyl was administered by gavage to female Sprague Dawley rats (23/dose) at 0, 100, 300 or 1000 mg/kg bw/d in 0.5% methyl cellulose, on days 6-15 of gestation (Anon. 1987, RAR Vol.3, B.6.6.2.1/02). Maternal findings were noted at 1000 mg/kg bw/d and included a 27% lower maternal body weight gain during GD6-11 compared to controls, as well as a 14% lower net body weight change (statistically significant negative trend). The number of corpora lutea, implantations and resorptions were not affected upon treatment. The only foetal effect observed was an increased foetal (litter) incidence of unossified 5th and 6th sternebrae at 1000 mg/kg bw/d. The DS considered this delayed ossification secondary to the maternal toxicity observed at this dose, thereby not warranting classification.

Rabbit

In a non-GLP developmental toxicity study using an in-house (OECD 414-like) method (Anon. 1991, RAR Vol.3, B.6.6.2.1/03), tolclofos-methyl was administered by gavage to female New Zealand White rabbits (13-17/dose) at 0, 300, 1000 or 3000 mg/kg bw/d in 0.5% carboxymethyl cellulose, on days 6-18 of gestation. One dam at 3000 mg/kg bw/d died on GD14. Abortions were observed in two dams at 3000 mg/kg bw/d (on GD20 and GD22) and in one dam at 1000 mg/kg bw/d (on GD26). Reductions in body weight (8-12%) were observed from 300 mg/kg bw/d. At 1000 and 3000 mg/kg bw, food consumption was reduced (during the first week of treatment), as well as body weight gain during GD6-19 (56 and 76%, respectively), kidney weight (11-13%) and spleen weight (20%, at 3000 mg/kg bw/d only). All foetal and skeletal and visceral observations were unaffected by treatment. Although considered adverse, the DS found the abortions were not sufficient for classification as the incidence was very low and observed only at and above the limit dose of 1000 mg/kg bw/d.

Overall, the DS concluded on the basis of the available studies in rats and rabbits that classification is not needed for effects on development.

Adverse effects on or via lactation

The DS did not propose classification for effects on or via lactation because the chemical does not meet the criteria, for two out of three criteria due to lack of data (i.e., there is no human evidence available indicating a hazard to babies, and the ability of tolclofos-methyl to distribute into the breast milk has not been investigated). Concerning the third criterion, the DS noted that in the rat one-generation reproduction study pup weights were reduced at all doses, but during lactation only at levels that were maternally toxic (5000 and 10000 ppm). Taking this into account, the DS considered the effect on bodyweight growth not to provide clear evidence for an adverse effect of tolclofos-methyl in the offspring due to transfer into the milk.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC considers the negative results in the rat three-generation reproduction study and the 1979 developmental toxicity study to not fully inform on the reproductive properties of tolclofos-methyl, given the low doses tested in these studies. RAC notes that higher, more appropriate doses have been tested in the rat one-generation reproduction study and in the 1987 and 1991 developmental toxicity studies in rats and rabbits, respectively.

In view of the absence of findings on fertility parameters in the one-generation reproduction study and the absence of adverse effects on the reproductive organs in this and other repeated dose studies, RAC agrees with the DS conclusion that tolclofos-methyl **does not need to be classified for effects on fertility and sexual function**.

RAC considers the delayed ossification observed in the 1987 rat developmental toxicity and the abortions noted in the rabbit developmental toxicity **do not warrant**

classification for developmental toxicity, for the reasons specified by the DS.

Likewise, RAC agrees with the conclusion of the DS that tolclofos-methyl **does not need to be classified for effects on or via lactation**.

10.11 Specific target organ toxicity-single exposure

Table 49: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral In house method in accordance with 92/69/EEC B.1 Rat Sprague Dawley M, F 10/sex/dose GLP: No	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil 1000, 2500, 3750, 5000 mg/kg bw Observations in 14 days	LD_{50}: >5000 mg/kg bw (M, F)	RAR Vol 3 B.6.2.1/01
Acute oral In house method in accordance with 92/69/EEC B.1 Rat Sprague Dawley F 10/dose GLP: Yes	Tolclofos-methyl (purity: 98.6%) Vehicle: maize oil 200, 5000 mg/kg bw (single dose) 2 mg/kg bw/day (dose given on four occasions two hours apart) formulated at 0.005%, 2% and 50% in maize oil Animals killed 8 hrs after the dosing.	LD_{50}: >5000 mg/kg bw (fasted F)	RAR Vol 3 B.6.2.1/02
Acute oral FIFRA § 81-1 Rat Sprague Dawley M, F 5/sex/dose No GLP but a statement of QA	Tolclofos-methyl (purity: 97.7%) 5000 mg/kg bw Observations in 14 days	LD_{50}: >5000 mg/kg bw (M, F)	RAR Vol 3 B.6.2.1/03
Acute oral In house method in accordance with 92/69/EEC B.1 Mouse dd M, F 10/sex/dose GLP: No	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil 1000, 1500, 2000, 3000, 4000 mg/kg bw Observations in 14 days	LD_{50}: 3500 mg/kg bw (M) 3600 mg/kg bw (F)	RAR Vol 3 B.6.2.1/01

Acute oral (gelatine capsules) In house method in accordance with 92/69/EEC B.1 Dog Beagle M, F 2/sex/dose GLP: No Considered as supplementary data	Tolclofos-methyl (purity: 98.7%) 100 and 1000 mg/kg bw (animals fasted) 215 and 464 mg/kg bw (animals unfasted) Observations in 16 days	LD_{50}: >1000 mg/kg bw (M, F) A suppression of brain cholinesterase activity in the high-dose (1000 mg/kg) animals was considered treatment-related (brain cholinesterase reduced 25% and 24% in males and females, respectively compared to low dose animals)	RAR Vol 3 B.6.2.1/04
Acute dermal In house method in accordance with 92/69/EEC B.3 Rat Sprague Dawley M, F 10/sex/dose GLP: No	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil 1000, 2500, 5000 mg/kg bw Observations in 14 days	LD_{50}: >5000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/01
Acute dermal OECD TG 402 Rat Sprague Dawley (Crl:CD(SD)) M, F 5/sex/dose GLP: Yes	Tolclofos-methyl (purity: 97.5%) 2000 mg/kg bw Observations in 14 days	LD_{50}: >2000 mg/kg bw	RAR Vol. 3 B.6.2.2/03
Acute dermal In house method in accordance with 92/69/EEC B.3 Mouse dd M, F 10/sex/dose GLP: No	Tolclofos-methyl (purity: 97.0%) Vehicle: corn oil 1000, 2500, 5000 mg/kg bw Observations in 14 days	LD_{50}: >5000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/01
Acute dermal FIFRA § 81-1 Rabbit New Zealand White M, F 5/sex/dose No GLP but a statement of QA	Tolclofos-methyl (purity: 97.7%) 2000 mg/kg bw Observations in 15 days	LD_{50}: >2000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/02

Acute inhalation In house method in accordance with 92/69/EEC B.2 Rat Wistar M, F 5/sex/dose GLP: Yes *The study was checked for compliance with OECD TG 403. Following deviation was noted: The mass median aerodynamic diameter, MMAD, was outside the upper range recommended in the guideline* *Study limited since the MMAD was >4 µm*	Tolclofos-methyl (purity: 97.4%) Dust 52% of airborne particles were 5.5 µm or less in aerodynamic diameter 0, 1.35, 3.32 mg/L 4 hr, whole body 14 days observation	LC_{50}: >3.32 mg/L (maximum attainable concentration) (M, F)	RAR Vol. 3 B.6.2.3/01
Acute inhalation OPPTS 870.1300 Rat Sprague Dawley M, F 5/sex/dose GLP: Yes *The study was checked for compliance with OECD TG 403. Following deviation was noted: dose level used in this study was not the maximum attainable concentration*	Tolclofos-methyl (purity: 97.4%) Dust MMAD: 3.6 µm (geometric standard deviation: 2.2 µm) 2.07 mg/L (mean achived atmosphere concentration) 4 hr, nose-only 14 days observation	LC_{50}: >2.07 mg/L (M, F)	RAR Vol. 3 B.6.2.3/02
Acute neurotoxicity Dose range finding study Oral Guideline: not applicable Rat Crl:CD(SD) M, F 5/sex/dose GLP: Yes *Limited parameters investigated in the study* *Study accepted for dose selection but not for NOAEL setting*	Tolclofos-methyl (purity: 97.5%) Vehicle: Corn oil 1000 and 2000 mg/kg bw Single dose	<u>1000 mg/kg bw:</u> No treatment-related effects <u>2000 mg/kg bw:</u> -clinical signs (a single incidence of walking on tiptoes noted for one female)	RAR Vol. 3 B.6.7.1.1/01

Acute neurotoxicity Time to peak effect study Oral Guideline: not applicable Rat Crl:CD(SD) M, F 20 M, 20 F GLP: Yes *Time to peak effect study of the effects of tolclofos-methyl on cholinesterase in rats. Study not suitable for NOAEL setting (limited parameters investigated in study).*	Tolclofos-methyl (purity: 97.5%) Vehicle: Corn oil 2000 mg/kg bw Single dose	2000 mg/kg bw: No treatment-related effects	RAR Vol 3 B.6.7.1.1/02
Acute neurotoxicity Oral (gavage) OECD TG 424 Rat Crl:CD(SD) M, F 12/sex/dose GLP: Yes	Tolclofos-methyl (purity: 97.5%) Vehicle: Corn oil 0, 200, 700, 2000 mg/kg bw Single dose	200 mg/kg bw: No treatment-related effects 700 mg/kg bw: ↓locomotor activity on study Day 0 (M, F) 2000 mg/kg bw: ↓locomotor activity on study Day 0 (M, F) NOAEL: 200 mg/kg bw/day	RAR Vol. 3 B.6.7.1.1/03

Table 50: Summary table of human data on STOT SE

No data

Table 51: 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

The acute toxicity studies are summarised in section 10.2.1. The single dose neurotoxicity studies are thoroughly presented in Vol. 3 to the RAR.

Following acute oral administration in rats toxic symtoms such as decrease of spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body developed 3-4 hrs after administration. Deaths were also noted at 5000 mg/kg bw. The toxic signs of surviving animals disappeared in 3 to 8 days. The minimum toxic dose level was 3750 mg/kg bw. No gross necropsy findings were observed. In mice the toxic symptoms were stated to be similar to those of rats and deaths were noted at 1500 mg/kg bw. The minimum toxic dose level was 1500 mg/kg bw. No gross necropsy findings were observed (RAR Vol. 3, B.6.2.1/01). In dogs clinical signs such as emesis were noted after a

single dose oral administration at ≥464 mg/kg bw, and stools or diarrhea were observed at ≥100 mg/kg bw. Furthermore, reduced brain cholinesterase activity was noted in dogs at 1000 mg/kg bw (males: 25% compared to 100 mg/kg bw group; females: 24% compared to 100 mg/kg bw group). No gross necropsy findings were observed (RAR Vol. 3, B.6.2.1/04).

Following acute dermal administration in rat and mice at the dose level of 5000 mg/kg bw no remarkable symptoms were noted (RAR Vol. 3, B.6.2.2/01). There were no dermal reaction or clinical signs observed in rabbits dermally exposed to tolclofos-methyl up to 2000 mg/kg bw. One female animal showed piloerection but completely recovered by day 14 (RAR Vol. 3, B.6.2.2/02).

Following acute inhalation administration in rats at a concentration of 2.07 mg/L all rats exhibited irregular respiration, but recovered from this symptom by day 3. Two of the male animals showed dry rales after removal from the exposure chamber up to a post-expsure period of 2.5 hrs (RAR Vol. 3, B.6.2.3/01).

Following acute subcutaneous administration in rats at the dose level of 5000 mg/kg bw no remarkable symptoms were noted. In the case of mice, the toxic symptoms such as decrease of spontaneous motor activity, piloerection, loss of appetite, irregular respiration and slight motor ataxia were developed 3 to 4 hrs after administration, at the dose level of 2000 mg/kg bw and above. At higher dose level of 4000 mg/kg bw and above, death of mice was observed within 24 hrs post-treatment and the mortality was 20% and 30% for male (4000 mg/kg bw) and female (5000 mg/kg bw), respectively. The toxic signs of surviving animals disappeared in 3 to 5 days. In the necropsy, the formation of granulation tissues and/or residues of oily substance were found in subcutaneous injection site of rats and mice (RAR Vol. 3, B.6.8.1.1/01).

Following acute intraperitoneal toxicity the toxic symtoms in rats were similar to those in the case of oral administration (decrease of spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body, B.6.2.1/01). The onset of toxic signs developed with 2 to 3 hrs post-treatment, at the dose level of 2000 mg/kg bw and above. Death of animals was observed 2 to 7 days after administration, and the toxic signs of surviving animals disappeared in 3 to 10 days. In mice, the LD_{50} values were 1070 and 1260 mg/kg bw for male and female mice, respectively, and the minimum toxic dose level was 650 mg/kg bw for both sexes. The toxic symptoms of mice treated intraperitoneally with tolclofos-methyl were essentially similar to those in rats. No remarkable changes were found at macroscopic examination (RAR Vol. 3, B.6.8.1.1/01).

In the time to peak effect study, tolclofos-methyl in corn oil as vehicle, was administered orally by gavage as a single dose (2000 mg/kg bw) to 20 male and 20 female Crl:CD(SD) rats. The purpose of the study was to determine the time at which peak inhibition of cholinesterase occurs in plasma, red blood cell (RBC) and whole brain homogenates. Cholinesterase activity was unaffected in the study, and no functional deficits were noted (RAR Vol. 3, B.6.7.1.1/02).

In the acute neurotoxicity study, the only finding after a single oral administration of tolclofos-methyl to rats at dose levels of 0, 200, 700 and 2000 mg/kg bw was decreased locomotor activity (total and ambulatory counts) noted for males and females in the 700 and 2000 mg/kg bw groups on study day 0 but not on days 7 and 14 (RAR Vol. 3, B.6.7.1.1/03).

In the dose-range finding study in the rat no treatment-related effects were noted except a single incidence of walking on tiptoes noted for one female in the 2000 mg/kg group. Based on the study results, dose levels of 200, 700 and 2000 mg/kg bw were selected for evaluation of the acute neurotoxic potential of tolclofos-methyl (RAR Vol. 3, B.6.7.1.1/01).

10.11.2 Comparison with the CLP criteria

According to the CLP Guidance, specific target organ toxicity (single exposure) 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. Regulation EC No 1272/2008 (CLP), Annex 1: 8.2.1.7.3, states for STOT SE: "*…Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, must be taken into consideration in the classification process, including but not limited to the following effects in humans and/or animals:…(b) Significant functional changes, more than transient in nature, in the respiratory system, central or peripheral nervous systems, other organs or other organ systems, including signs of central nervous system depression and effects on special senses (such as sight, hearing and sense of smell)…"*

According to the CLP Guidance Table 3.8.1, following criteria should be fulfilled for a classification in Category 1 or 2:

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure Substances are classificed in Category 1 for specific target organ toxicity (single exposure) on the basis of: a. reliable and good quality evidence from human cases or epidemiological studies; or b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be use as part of weight-of-evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure. Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) in order to help in classification In exceptional cases, human evidence can also be used to place a substance in Category 2 (see 3.8.2.1.6)

STOT-SE Category 1 and 2 is assigned on the basis of findings of "significant" or "severe" toxicity. In this context "significant" means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound or serious than "significant" effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

Guidance values ranges for single-dose exposures are given below:

Route of exposure	Units	Category 1	Category 2	Category 3
Oral (rat)	mg/kg bw	C≤300	2000≥C>300	Guidance values do not apply
Dermal (rat or rabbit)	mg/kg bw	C≤1000	2000≥C>1000	
Inhalation (rat) gas	ppmV/4h	C≤2500	20000≥C>2500	
Inhalation (rat) vapour	mg/l/4h	C≤10	20≥C>10	
Inhalation (rat) dust/mist/fume	mg/l/4h	C≤1.0	5.0≥C>1.0	

The criteria for classification in Category 3 only cover the transient effects of "respiratory tract irritation" and narcotic effects"

According to the CLP Guidance Table 3.8.1, following criteria should be fulfilled for a classification in Category 3:

Category 3	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects as laid down in 3.8.2.2

Annex 1: 3.8.2.2.1 Criteria for respiratory tract irritation

The criteria for classifying substances as Category 3 for respiratory tract irritation are:

a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.

b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids).

c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of "irritation" shall be excluded as this term is commonly

used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation , and dryness, shich are outside the scope of classification for respiratory irritation.

d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.

e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

In the acute toxicity studies performed on tolclofos-methyl, no specific target organ toxicity were noted in rats and mice. Reduced brain cholinesterase activity were noted in dogs (males: 25% reduction compared to the low dose animals; females: 24% reduction compared to the low dose animals) at the dose level of 1000 mg/kg bw. The study was considered as supplementary data only (no GLP, no statistical analysis conducted and low number of animals used). Therefore the study seems not to be well-substantiated data for classification.

In the time to peak effect study, cholinesterase activity was unaffected following a single dose administration (2000 mg/kg bw) in Crl:CD(SD) rats, and no functional deficits were noted. Thus the criteria for classification in Cat 1 or 2 is not fulfilled.

In the acute neurotoxicity study decreased locomotor activity (total and ambulatory counts) was noted for male and female rats in the 700 and 2000 mg/kg bw groups on study day 0 but not on days 7 and 14. Thus the effect was transient in nature and not considered adverse for classification in Cat 1 or 2.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification is proposed for tolclofos-methyl.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier Submitter's proposal There are four oral acute toxicity studies (rats/mice/dogs), three dermal acute toxicity studies (rats/mice/rabbits) and two acute inhalation toxicity studies (rats) available investigating the effects of a single dose of tolclofos-methyl. The results of these studies have been described in detail in the section on 'Acute toxicity' above. In addition, three acute oral neurotoxicity studies (rats) were presented in the CLH dossier.

In a dose-range finding acute neurotoxicity study (Anon. 2010, RAR Vol.3 B.6.7.1.1/01; GLP compliant), Crl:CD(SD) rats (5/sex/dose) were treated by gavage with a single tolclofos-methyl dose of 0, 1000 or 2000 mg/kg bw (vehicle corn oil). No mortalities or treatment-related effects were noted except for a single incidence of walking on tiptoes for one female in the 2000 mg/kg bw group.

In the main (GLP and OECD 424 compliant) acute neurotoxicity study (Anon. 2010, RAR Vol.3 B.6.7.1.1/03), tolclofos-methyl in corn oil was administered by gavage as a single dose of 0, 200, 700 or 2000 mg/kg bw to Crl:CD(SD) rats (12/sex/dose). The only finding was decreased locomotor activity (total and ambulatory counts) in males and females of the 700 and 2000 mg/kg bw groups on study day 0, but not on days 7 and 14.

In another GLP compliant acute neurotoxicity study (Anon. 2010, RAR Vol.3 B.6.7.1.1/02), Crl:CD(SD) rats (20/sex/group) were treated by gavage with a single tolclofos-methyl dose of 0 or 2000 mg/kg bw (vehicle corn oil). This study was aimed at determining the time at which peak inhibition of cholinesterase occurs in plasma, red blood cell and whole brain homogenates. In this study 5 animals/sex/group/time point were euthanized at 1, 2, 4 and 8 hours after dosing. No mortalities and no clinical signs of toxicity were observed. No effect on cholinesterase activity was noted and there were no functional deficits.

Based on the results of the available acute toxicity studies with tolclofos-methyl, the DS considered that no specific toxic effects on organs were noted in rats or mice. As to neurotoxic effects, the DS considered the effects in rats (only a transient decrease in locomotor activity was observed) not sufficiently adverse for classification in category 1 or 2, particularly since acetylcholinesterase activity was unaffected and no functional deficits were noted in a time to peak effect study. Although a greater than 20% inhibition was observed for brain acetylcholinesterase activity in dogs, the DS considered the dog study not well-substantiated data for classification given its non-GLP status, the limited number of animals used and the lack of statistical analysis. The DS therefore proposed no classification for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the standard acute toxicity studies in rats, mice, dogs and rabbits, treated animals showed a variety of clinical signs, most of which considered to be indicative of general, non-specific toxicity and not fulfilling the criteria for classification with STOT SE 1 or 2. Measurement of acetylcholinesterase activity was included in two acute toxicity studies in rats and in one acute toxicity study in dogs. In rats, tolclofos-methyl did not affect brain, plasma or erythrocyte acetylcholinesterase activity following single doses up to and including 5000 mg/kg bw. In dogs, however, a single dose of 1000 mg/kg bw tolclofos-methyl caused a 25%/24% (m/f) inhibition of brain acetylcholinesterase activity compared to a single dose of 100 mg/kg bw. Erythrocyte and plasma acetylcholinesterase activity were unaffected, and no clinical signs indicative of neurotoxicity were noted in the dogs at 1000 mg/kg bw. RAC notes the low number of animals in this study (2/sex/dose), the lack of statistical analysis, as well as the absence of a dose-relation in

the inhibition (brain acetylcholinesterase activity at 215 and 464 mg/kg bw was about equal or even higher than that at 100 mg/kg bw). RAC therefore agrees with the DS that classification with STOT SE 1 or 2 is not warranted for the effect on brain acetylcholinesterase activity in dogs. RAC also agrees with the DS that the transient decrease in locomotor activity observed in an acute oral neurotoxicity study in rats is not sufficient to warrant classification with STOT SE 1 or 2.

Classification for STOT SE 3 is also not warranted, as no signs of respiratory tract irritation were observed in the acute studies available, and the depression of motor activity observed in the acute neurotoxicity study, despite being transient, does not fulfil the criteria for narcotic effects.

RAC therefore agrees with the conclusion of the DS that tolclofos-methyl **should not be classified for specific target organ toxicity – single exposure (STOT SE)**.

10.12 Specific target organ toxicity-repeated exposure

Table 52: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
4-week Oral (dietary) In-house method, in accordance with 92/69/EEC, B.7. Rat CD rats of Sprague Dawley strain M, F 10/sex/dose GLP: Yes *The study report was checked for compliance with OECD TG 407 adopted 3 October 2008 and the following deviations were observed: No detailed clinical observations were made in the animals, no functional observations were conducted, oestrus cycle of females were not determined, haematocrit and blood clotting time/potential were not determined, urinalysis was not performed, the endocrine activity was not determined, epididymides, prostate, thymus were not weighed, epididymides and vagina were not examined microscopically*	Tolclofos-methyl (purity: not specified) 0, 200, 1000, 5000, 20000 ppm (corresponding to 0, 16, 79, 414, 1635 mg/kg bw/day in males; 0, 18, 88, 452, 1830 mg/kg bw/day in females)	<u>200 ppm:</u> ↓brain cholinesterase activity (M: 12%) ↓erythrocyte cholinesterase activity (M: 10%) <u>1000 ppm:</u> ↓brain cholinesterase activity (M: 18%) -organ weight changes (↑kidney weight (M) rel: 14%, abs: 9%) <u>5000 ppm:</u> ↓brain cholinesterase activity (M: 17%; F: 20%) ↓erythrocyte cholinesterase activity (M: 18%; F: 19%) - organ weight changes (↑kidney weight (M: rel: 10%, abs: 12%, F: rel: 8%, abs: 13%), ↑liver (F: rel: 7%, abs: 12%)) <u>20000 ppm:</u> -clinical sign (patchy hair loss) (F) ↓bw gain (M: 45%, F: 37%) ↓FC (M, F) - changes in biochemical parameters (↑albumin (M), ↑total protein (M), ↑inorganic phosphorus (F)) ↑cholesterol (M,F) ↓brain cholinesterase activity (M: 31%; F: 21%) ↓plasma cholinesterase activity (M: 14%; F: 39%) ↓erythrocyte cholinesterase activity (M: 19%) -organ weight changes (↑liver weight (M: rel: 3% abs: 27%, F: rel: 15%, abs: 39%), ↑rel kidney weight (M: rel: 8%, abs: 12%)) - histopathological changes (hepatocyte enlargement (M,F))	RAR Vol. 3, B.6.3.1.1/01

		NOAEL M: 5000 ppm (corresponding to 414 mg/kg bw/day) NOAEL F: 1000 ppm (equivalent to 88 mg/kg bw/day)	
90-day Oral (dietary) FIFRA §82-1 Rat Crj:CD(SD) M, F 12/sex/dose GLP: Yes *The study report was checked for compliance with OECD TG 408 adopted 21 September 1998 and the following deviations were observed: No functional observations were conducted, epididymides and uterus were not weighed, volume and osmolality were not determined.*	Tolclofos-methyl (purity: 96.6%) 0, 100, 1000 and 10000 ppm (corresponding to 0, 6.46, 66.1 and 653 mg/kg bw/day for males; 0, 7.13, 71.0 and 696 mg/kg bw/day for females)	<u>100 ppm:</u> -changes in biochemical parameters (↓glutamic-oxaloacetictransaminase (M)) <u>1000 ppm:</u> -changes in biochemical parameters (↑α2-globulin (M), ↑inorganic phosphorus (F), ↓γ-globulin (F), ↓glucose (F) ↓erythrocyte cholesterase (F:10%) -organ weight changes (↑rel liver (M, 4%), ↑relative kidney (F, 10%)) <u>10000 ppm:</u> -clinical sign (loss of hair) (1M, 3F) ↓bw (M: 19%, F: 15%) ↓bw gain (M: 25%, F: 27%) ↓FC (M, F) -changes in haematological parameters (↑activated partial thromboplastin time (M), ↓haemoglobin concentration (F, 3%), ↓mean corpuscular haemoglobin (F, 3%), ↓prothrombin time (F, 2%), ↑total leukocyte count (F), ↑lymphocyte count (F)) -changes in biochemical parameters (↑total protein (M), ↑α2-globulin (M, F), ↑ β-globulin (M, F), ↑total cholesterol (M, F), ↑phospholipid (M, F), ↑calcium (M, F), ↑ inorganic phosphorus (M, F), ↑triglycerides (F), ↑ γ-glutamyl transpeptidase (M), ↑blood urea nitrogen (M), ↓γ-globulin (F), ↓glucose (F), ↓alkaline phosphatase (F), ↓triglycerides (M) ↓erythrocyte cholinesterase values (M: 17%; F: 20%) ↓plasma cholinesterase values (M: 17%; F: 53%) ↓brain cholinesterase values (M: 8%; F: 9%)	RAR, Vol. 3, B.6.3.2.1/01

		-changes in urinalysis parameters (↓PH) (M, F) -organ weight changes (↑rel liver (M: 34%, F: 37%), ↑abs liver (F: 16%), ↑relative kidney (M: 15%, F: 25%)) -macroscopic changes in the liver (dark-red discoloration, M, F) -histopathological changes in the liver (hypertrophy, M, F) NOAEL M, F: 1000 ppm (corresponding to 66.1 and 71.0 mg/kg bw/day in males and females, respectively)	
6-month Oral (dietary) In-house method Rat Sprague Dawley M, F 15/sex/dose No GLP but a QA statement *The study report was checked for compliance with OECD TG 409 adopted 21 September 1998 and the following deviations were observed: No functional observations were conducted, platelet count and a measure of blood clotting time/potential were not investigated, cholesterol and creatinine were not investigated, epididymides, uterus and thymus were not weighed, aorta, mammary gland and peripheral nerve were not examined microscopically*	Tolclofos-methyl (purity: 97%) 0, 300, 1000, 3000 and 10000 ppm (corresponding to 0, 16, 51, 164 and 540 mg/kg bw/day for males; 0, 18, 65, 184, 623 mg/kg bw/day for females)	<u>300 ppm:</u> No treatment related effects (increased rel weight of kidney (11%) in females was not considered treatment related because of the correlation between the kidney weight and the body weight) <u>1000 ppm:</u> -changes in organ weights (↑abs (10%) and rel liver (8%) (F),↑abs (25%) and rel kidneys (24%) (F)) <u>3000 ppm:</u> ↓erythrocyte cholinesterase activity (M: 17%) -changes in biochemical parameters (↓uric acid) (F) -changes in organ weights (↑abs (12%) and rel liver (13%) (F), ↑abs (21%) and rel kidneys (22%) (F)) <u>10000 ppm:</u> ↓bw (M: 8% n.s, F: 15%) ↓bw gain (M: 6% n.s, F: 18%) -changes in haematological parameters (↓Hb concentration 5% (F)) -changes in biochemical parameters (↓uric acid concentration (F)) ↓plasma cholinesterase activity (F: 23-40% during the first 3 months) ↓erythrocyte cholinesterase activity (M: 19%) -changes in organ weights (↑rel liver (M: 10%, F:21%), ↑rel kidney (M:20%, F:37%), ↑abs kidney (F: 17%), ↑rel testes:12%)	RAR, Vol. 3, B.6.3.3.1/01

		NOAEL M: 3000 ppm (corresponding to 164 mg/kg bw/day) NOAEL F: 300 ppm (corresponding to 18 mg/kg bw/day)	
9-month Oral (dietary) EPA Guideline 82-1 Mouse ddY M, F 15/sex/dose GLP: No *The study was checked for compliance with OECD TG 408 and following deviations were noted: No functional observations were conducted, observations of sensory reactivity to stimuli of different types, assessment of grip strength and motor activity assessment were not conducted, blood clotting time/potential was not determined, sodium and potassium were not determined, epididymides and thymus were not weighed, volume and osmolality were not determined, parathyroid, aorta mammary gland, gall bladder, lymph node were not examined microscopically*	Tolclofos-methyl (purity: 97%) 0, 10, 30, 100 and 3000 ppm (corresponding to 0, 1.2, 3.8, 12.2 and 513 mg/kg bw/day for males and 0, 1.4, 4.1, 13.8 and 564 mg/kg bw/day for females)	<u>10 ppm:</u> ↓plasma cholinesterase activity (F: 24%) <u>30 ppm:</u> ↓plasma cholinesterase activity (F: 37%) <u>100 ppm:</u> ↓plasma cholinesterase activity (M: 44%, F: 58%) ↓erythrocyte cholinesterase activity (M: 20%, F: 13%) <u>3000 ppm:</u> ↓bw (M,F) (M: 21%, F: 23%) ↓bw gain (M,F) (M: 33%, F: 30%) -changes in biochemical parameters (↑cholesterol concentration) (F) ↓plasma cholinesterase activity (M: 95%, F: 88%) ↓erythrocyte cholinesterase (M; 55%, F: 35%) ↓brain cholinesterase activity (M: 25%) -changes in organ weights (↑rel heart (M, F), ↓ abs heart (M, F), ↑rel testis, ↑ rel liver (M: 11%, F: 10%), ↓abs liver (M: 12%, F:19%), ↑rel kidney (M: 21%) ↓abs kidney (F: 20%), ↑rel brain (M, F), ↑rel lung (M), ↑rel adrenal (M: 22%, F: 29%), ↑rel pituitary (M: 62%, F: 78%), ↑abs pituitary (F: 37%)) NOAEL M: 30 ppm (corresponding to 3.8 mg/kg bw/day) NOAEL F: 100 ppm (corresponding to 13.8 mg/kg bw/day)	RAR Vol. 3, B.6.3.4.1/01
6-month Oral (dietary) In-house method	Tolclofos-methyl (purity: 98.7%) 0, 200, 600 and 2000 ppm (corresponding to 0, 6.6, 24, 70 mg/kg bw/day for males; 0, 6.0, 21, 63 mg/kg bw/day for females)	<u>200 ppm:</u> No treatment-related effects <u>600 ppm:</u> -changes in haematological parameters (M: reduced Hb at	RAR Vol. 3, B.6.3.3.2/01

Dog Beagle M, F 6/sex/dose GLP: No *The study was checked for compliance with the OECD TG 408 adopted 21 September 1998 and the following deviations were observed: Observations of sensory reactivity to stimuli of different types, assessment of grip strength and motor activity assessment were not conducted, no functional observations were conducted, blood clotting time/potential was not determined, sodium and potassium were not determined, epididymides and thymus were not weiged, volume and osmolality were not determined, parathyroid, aorta, mammary gland, gall bladder, lymph node were not examined microscopically*		week 12: 11%, at week 16: 8%) <u>2000 ppm:</u> -changes in haematological parameters (M: ↓Hb at week 12: 14%; week 24: 13%↓ erythrocyte count); F: ↓Hb at week 20: 11%,↓Hb at week 23: 14%, ↓hematocrit at week 20: 10%, ↓erythrocyte count) -changes in biochemical parameters (↑alkaline phosphatase) (M,F) ↓plasma cholinesterase (19%) (F) -changes in organ weights (↑abs and rel liver) (M: rel: 79%, abs: 56%, F: rel: 65%, abs: 43%) NOAEL: 600 ppm (24 and 21 mg/kg bw/day in males and females, respectively)	
1-year Oral (dietary) EPA Guideline 83-1 Dog Beagle M, F 6/sex/dose GLP: Yes *The study was checked for compliance with the OECD TG 409 and the following deviations were noted: General clinical observations were made. It is not indicated in the study if changes in gait, posture and response to handling as well as the presence of clonic or tonic movements were recorded.*	Tolclofos-methyl (purity: 97.6%) 0, 80, 400 and 2000 ppm (equivalent to 0, 2.2, 11.4 and 59 mg/kg bw/day for males and 0, 2.6, 11.2 and 62 mg/kg bw/day for females)	<u>80 ppm:</u> No treatment-related effects <u>400 ppm:</u> -histopathological changes (slight hepatocytic pigment) (M, F) <u>2000 ppm:</u> ↓bw (M,F) (M: 13% n.s.; F: 16% n.s.) ↓bw gain (M, F) (M:week 0-52: 32%, n.s.; F: week 0-26: 65% (s.s.), week 0-52: 54% (n.s.)) ↓FC (F) -changes in haematological parameters (↓erythrocyte count, ↓ haematocrit, ↓haemoglobin (M:17% (s.s.); F: 8% (n.s.)), ↑platelet count (M)) -changes in biochemical parameters (↓albumin (s.s in F only),↓ total protein (s.s. in F only), ↑alkaline phosphatase values (s.s) (M, F) -changes in urinalysis (increased incidence and severity of reducing substances) (M, F) -changes in organ weights (↑abs and rel liver (M: rel: 74%, abs: 61%, F: rel: 52%, abs: 32%), ↑rel pancreas (M: 27%, F: 50%), ↓abs prostate 45%)	RAR Vol. 3, B.6.3.5.1/01

		-macroscopic changes (liver: dark in colour (M, F); prostate: small in size) -histopathological changes (hepatocytic hypertrophy, intracytoplasmic homogenous material and heptatocytic pigment) (M,F) NOAEL M, F: 400 ppm (corresponding to 11.4 and 11.2 mg/kg bw/day in males and females, respectively)	
Dermal 21-day EPA Guideline 158, 82-2 Rabbit New Zealand White/Hazleton Dutchland M, F 5/sex/dose GLP: Y	Tolclofos-methyl (purity: 97.7%) Vehicle: Acetone 0, 30, 300 and 1000 mg/kg bw/day	<u>30 mg/kg bw/day:</u> -dermal irritation (erythema) (M, F) -histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration) (M, F) <u>300 mg/kg bw/day:</u> -dermal irritation (erythema) (M, F) -↓plasma cholinesterase activity (F: 29%) -histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration) (M, F) <u>1000 mg/kg bw/day:</u> -dermal irritation (erythema) (M, F) -changes in haematological parameters (↑eosinophil value) (M) -↓plasma cholinesterase activity (F: 25%) -changes in organ weights (↑rel kidney, 20%) (F) -histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration) (M, F) NOAELsystemic M: ≥1000 mg/kg bw/day NOAELsystemic F: 300 mg/kg bw/day NOAEL local effects M, F: not determined	RAR Vol. 3, B.6.3.6/01
Subchronic neurotoxicity Oral (dietary) OECD TG 424	Tolclofos-methyl (purity: 96.8%) 0, 300, 1800, 10000 ppm (main	Main study: <u>300 ppm:</u> No treatment-related effects	RAR, Vol. 3, B.6.7.1.2/01

Rat Alpk:APfSD (Wistar-derived) M, F 12/sex/dose (main study) 5/sex/dos (satellite group for acetyl cholinesterase measurements) GLP: Yes	phase). Equivalent to 0, 20.6, 122.3 and 735.7 mg/kg bw/day in males; 0, 23.1, 135.8 and 762.7 mg/kg bw/day in females 300, 1800 and 10000 ppm (satellite group). Equivalent to 22.6, 130.2, 719.7 mg/kg bw/day in males; 24.3, 143.9, 817.5 mg/kg bw/day in females	<u>1800 ppm:</u> No treatment-related effects <u>10000 ppm:</u> ↓bw (M: 17%, F: 11%) ↓bw gain (At week 14: M: 31%, F: 18%) ↓FC (M, F) ↓brain weight (week, 14, M) Satellite study: <u>300 ppm:</u> ↓erythrocyte cholinesterase (F: 8% (week 14) <u>1800 ppm:</u> ↓brain cholinesterase (F: 13% (week 14) ↓erythrocyte cholinesterase (M: 10% (week 14), F: 14% (week 14)) <u>10000 ppm:</u> ↓bw (M: 17%, F: 12%) ↓bw gain (at week 9: M: 39%, F: 30%) ↓brain cholinesterase (M: 14% (week 5), F: 11% (week 2), 14% (week 14)) ↓erythrocyte cholinesterase (M; 24% (week 5), 17% (week 9), 18% (week 14), F: 14% (week 9), 15% (week 14)) ↓brain weight (week 9, F) (week, 14, M) NOAEL M, F: 1800 ppm (122.3 and 135.8 mg/kg bw/day in males and females, respectively)	
Developmental toxicity Oral (gavage) In house method Rat Fischer 344 CD®F F 30/dose GLP: No *Low dose levels were used in the*	Tolclofos-methyl (purity: 94.9%) Vehicle: 0.5% methylcellulose 0, 5, 15, 50 mg/kg bw/day Gestation Days 6-15	Maternal effects: <u>5 and 15 mg/kg bw/day:</u> No treatment-related effects <u>50 mg/kg bw/day:</u> ↓ mean implantation efficiency (86.1% compared to 91.9% in control group) Developmental effects: <u>5, 15 and 50 mg/kg bw/day:</u>	RAR, Vol. 3, B.6.6.2.1/01

study		No treatment-related effects NOAEL maternal and developmental: ≥50 mg/kg bw/day	
Developmental toxicity Oral (gavage) EPA 83-3 Rat Sprague Dawley F 23/dose GLP: No	Tolclofos-methyl (purity: 96.7%) Vehicle: 0.5% methylcellulose 0, 100, 300 and 1000 mg/kg bw/day Gestation Days 6-15	Maternal effects: <u>100 mg/kg bw/day</u> No treatment-related effects <u>300 mg/kg bw/day:</u> No treatment-related effects <u>1000 mg/kg bw/day:</u> ↓bw gain during Days 6-11 (27%) (net bodyweight change of 14% showed a statistically significant negative trend) Developmental effects: <u>100 mg/kg bw/day:</u> No treatment-related effects <u>300 mg/kg bw/day:</u> No treatment-related effects <u>1000 mg/kg bw/day:</u> - increased incidence of unossified 5th and 6th sternebrae NOAEL maternal: 300 mg/kg bw/day NOAEL developmental: 300 mg/kg bw/day	RAR Vol. 3, B.6.6.2.1/02
Developmental toxicity Oral (gavage) In house method Rabbit New Zealand White F 13-17/dose GLP: No *Few animals were used in study (OECD TG 414 recommends sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy)*	Tolclofos-methyl (purity: 98.7%) 0, 300, 1000, 3000 mg/kg bw/day Vehicle: 0.5% carboxymethylcellulose Gestation Days 6-18	Maternal effects: <u>300 mg/kg bw/day</u> ↓bw (Day 29: 10%) <u>1000 mg/kg bw/day:</u> -abortion (one dam on GD 26) ↓bw (Day 29: 11%) ↓bw gain (56%) ↓FC -organ weight changes (↓kidney 12%) <u>3000 mg/kg bw/day:</u> -mortality (one dam died on GD 14) -abortions (two dams on GD 20	RAR Vol. 3, B.6.6.2.1/03

		and 22, respectively) ↓bw (Day 29:11%) ↓bw gain (76%) ↓FC -organ weight changes (↓kidney 13%, ↓spleen 20%) Developmental effects: <u>300 mg/kg bw/day:</u> No treatment-related effects <u>1000 and 3000 mg/kg bw/day:</u> -abortions NOAEL maternal: 300 mg/kg bw/day NOAEL developmental: 300 mg/kg bw/day	
Three-generation Oral (dietary) In house method Rat Sprague Dawley CD albino M, F P1: 30/sex/group P2: 100 /sex/generation P3: 25/sex/group No GLP but a QA statement *Primordial follicles and sperm parameters(sperm morphology and motility) were not performed, sexual maturation was not investigated in study, prostate, seminal vesicles, pituitary, thyroid and uterus were not weighed. Histopathological examination was not performed for vaigna and epididymides. Dose levels were low.*	Tolclofos-methyl (purity: 98.7%) 0, 100, 300, 1000 ppm Equivalent to (during 15 weeks pre-mating): P1: 0, 6.9, 20.5, 70.6 mg/kg bw/day in males; 0, 8.9, 26.2, 90.5 mg/kg bw/day in females P2: 0, 7.9, 23.4, 79.6 mg/kg bw/day in males; 0, 9.2, 26.9, 98.5 mg/kg bw/day in females P3: 0, 7.6, 23.8, 78.2 mg/kg bw/day in males; 0, 9.0, 28.4, 96.1 mg/kg bw/day in females 15 weeks prior to mating and during mating, gestation and lactation.	<u>100 ppm:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is obscure, since the values fell within the normal range of the laboratory) <u>300 ppm:</u> <u>Parental:</u> ↓bw in P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is obscure, since the values fell within the normal range of the laboratory) <u>1000 ppm:</u> <u>Parental:</u> ↓bw in P2 and P3 males at week 4 after mating (4%, n.s.) <u>Offsprings:</u> No treatment related effects (There was a tendency towards increased percentages of male pups per litter during the study. The connection to treatment is	RAR, Vol. 3 B.6.6.1/01

		obscure, since the values fell within the normal range of the laboratory) NOAEL: Parental and pups: ≥1000 ppm (≥70.6 mg/kg bw/day) NOAEL: Reproductive: ≥1000 ppm (≥70.6 mg/kg bw/day)	
One-generation Oral (dietary) In house method Rat Crj:CD(SD)(SPF) M, F P: 10/sex/group F1: 10/sex/group GLP: No *Low number of animals in each group. Males treated 2 weeks prior to mating (OECD TG 415 recommends ten weeks prior to the mating period)*	Tolclofos-methyl (purity: 97.1%) 0, 2500, 5000, 10000 ppm. Equivalent to average intakes in the parental generation during pre-mating in males of 0, 173, 338 and 680 mg/kg bw/day and in females of 0, 178, 353, 668 mg/kg bw/day. For the pregnant and delivered dams average intakes were 0, 175, 341 and 642 mg/kg bw/day day during the gestational period, and 0, 360, 698 and 1253 mg/kg bw/day during the lactation period. In the offspring generation average intakes were 0, 255, 519 and 1161 mg/kg bw/day in males and 0, 257, 529 and 1174 mg/kg bw/day in females. <u>Parent females:</u> from 2 weeks prior to mating until autopsy after weaning throughout mating, gestation and lactation periods. <u>Parent males:</u> from 2 weeks prior to mating to autopsy. <u>F1 generation:</u> from weaning to autopsy at the age of 8 weeks.	<u>Parental</u> <u>2500 ppm:</u> -changes in organ weights (↑ abs liver (F: 8%)) <u>5000 ppm:</u> ↓bw (F: gestation period (Day 7: 5%), lactation period (5-9%)) ↓bw gain (F: lactation period 0-14 (43%)) ↓FC (F: middle to late stage of lactation period) ↓cholinesterase values in brain (M: 6%, F: 11%) -changes in organ weights (↑ abs liver (F:21%), (↑ rel liver (F:27%), ↑rel kidney (F:13%)) <u>10000 ppm:</u> ↓bw (M: early premating period (Week 1: 5%); F: gestation period (5-7%), lactation period (8-20%)) ↓bw gain (M: early premating period (Weeks 0-1: 33%), F: premating period (Weeks 0-1: 51%), lactation period (period 0-7: 84%, period 0-14: -8.6 g, period 0-21: -4.3 g)) ↓FC (F: early premating, gestation, lactation) ↓cholinesterase values in brain (M: 11%, F: 18%) -organ weight changes (↑rel brain (F), ↑ abs liver (M:17%, F:25%), ↑ rel liver (M:21%, F:51%), ↑rel kidney (M:9%, F:18%), ↓abs and rel ovaries, ↓abs and rel uterus) <u>Offsprings:</u> <u>2500 ppm:</u> ↓bw (F1 generation: M: 8% (PND 21) 9% (PND 28), F: 8% (PND 21 and 35) 7% (PND 42 and 56)) ↓bw gain (F1 generation: F: 14% (PND 21-49))	RAR, Vol. 3 B.6.6.1/02

		↓FC (M, F) -organ weight changes in F1 pups (↓abs spleen (M, F), ↓rel spleen (M)) -organ weight changes in F1 generation (↑rel liver (M: 9%), ↑rel kidney (F: 19%)) <u>5000 ppm:</u> ↓bw (F1 pups: M: 15% (PND 21), F: 17% (PND 21); F1 generation: M: 10% (PND 56), F: 11% (PND 56)) ↓bw gain (F1 pups: M: 16% (PND 0-21); F: 17% (PND 0-21); F1 generation: M: 9% (PND 21-56), F: 10% (PND 0-21)) ↓FC (M, F) -organ weight changes in F1 pups (↓abs brain (M), ↑ rel brain (M, F), ↓abs spleen (M, F) ,↓rel spleen (M, F)) -organ weight changes in F1 generation (↓abs brain (M, F), ↑rel liver (M:16%, F: 24%), ↑rel kidney (M:18%, F:21%), ↑rel testis) <u>10000 ppm:</u> ↓bw (F1 pups: M: 46%, F: 44%; F1 generation: M: 31% (PND 56); F: 24% (PND 56)) ↓bw gain (F1 pups: M: 50% (PND 0-21); F: 48% (PND 0-21); F1 generation: M: 27% (PND 21-56), F: 16% (PND 21-56)) ↓FC (M, F) -delayed preputial separation (preputial separation completed Day 46, compared to 43.6 in controls) -organ weight changes in F1 pups (↓abs brain (M, F), ↑ rel brain (M, F), ↓abs thymus (M, F), ↓rel thymus (M), ↓abs spleen (M, F), ↓rel spleen (M, F) ↓abs uterus -organ weight changes in F1 generation (↓abs brain (M, F), ↑ rel brain (M, F), ↑rel liver (M:39%, F:45%), ↓abs kidney (M:15%), ↑rel kidney (M:23%, F:22%), ↑rel testis, ↓abs ovary, ↓abs epididymides, ↑rel epididymides, ↓abs seminal vesicles, ↑rel prostate 25%)	

		NOAEL parental: 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) NOAEL offspring generation: not established. NOAEL reproductive toxicity: ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively)	
Combined two year chronic toxicity/carcinogenicity study in rats (Fischer 344 CD®F) In house method The study was checked for compliance with OECD 453. Following deviation was noted: males were exposed up to 122 weeks and females up to 129 weeks GLP: No	Tolclofos-methyl, 94.9% 0, 100, 300, 1000 ppm equivalent to 0, 4.2, 12, 42 mg/kg bw/day (males) 0, 4.8, 15, 49 mg/kg bw/day (females)	No distinct signs of compound effect were observed with regard to mortality, clinical signs, body weights, food consumption, organ weights and organ/body weight ratios, gross pathology and histopathology were attributable to the test compound at dietary levels up to 1000 ppm. Minor changes in clinical chemistry parameters (↓alkaline phosphatase) were noted in males of all treated groups. The high dose, 1000 ppm, represent a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies. NOAEL: ≥ 1000 ppm (≥42 mg/kg bw/day) Tolclofos-methyl was not oncogenic in this study.	RAR, Vol. 3, B.6.5.1/01
104-week cholinesterase activity study in male and female rats (Fischer 344) In house method GLP: No but performed according to GLP of US Food and Drug Administration	Tolclofos-methyl, 98.3% 0, 100, 300, 1000 ppm equivalent to 0, 4.1, 12, 42 mg/kg bw/day (males) 0, 4.8, 15, 49 mg/kg bw/day (females)	No treatment-related effects were shown on the plasma, erythrocyte or brain cholinesterase. The high dose, 1000 ppm, represents a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies. *The study is regarded as supplementary data*	RAR, Vol. 3, B.6.5.1/02
Combined two year chronic toxicity/carcinogenicity study in mice (Crj:B6C3F1) In house method Checked for compliance with OECD 453: Deviations:	Tolclofos-methyl, 94.3% 0, 10, 50, 250, 1000 ppm equivalent to 0, 1.3, 6.4, 32.2, 134 mg/kg bw/day (males) 0, 1.3, 6.9, 34.1, 137 mg/kg	No treatment related mortality, no haematology changes. Suppression of weight gain after 52 weeks in the female 1000 ppm group (15%) and decreased food consumption at week 104 in the	RAR, Vol. 3, B.6.5.2/01

prothrombin time, activated partial thromboplastin time, albumin, calcium, sodium, potassium, total cholesterol were not measured GLP: No	bw/day (females)	female 1000 group. Decrease in plasma, erythrocyte and brain cholinesterase at both sexes at 250 and 1000 ppm (At 250 ppm (week 104): plasma cholinesterase activity reduced 25% in males and 33% in females; erythrocyte cholinesterase activity reduced 13% in males and 11% in females, brain cholinesterase activity reduced 13% in males; At 1000 ppm: plasma cholinesterase activity reduced 43% in males and 59% in females, erythrocyte cholinesterase activity reduced 13% in males and 23% in females, brain cholinesterase activity reduced 26% in males and 9% in females). At week 28 in females, the cholinesterase activity was decreased already at 50 ppm (reduction in plasma (12%) and brain (18%)). At week 52 erythrocyte cholinesterase activity was reduced 23% in females and 18% in males at 250 ppm, and brain cholinesterase activity was reduced 12% in females and 14% in males at 250 ppm Glucose was increased in male 1000 ppm group after 104 weeks. Increase in kidney weight noted in both sexes at ≥250 ppm (250 ppm (week 52): ↑absolute weight 15% (males) ↑relative weight 9% (females); 250 ppm (week 104): ↑absolute weight 3% (females), ↑relative weight 11% (females); 1000 ppm (week 52): ↑absolute weight 15% (males), ↑relative weight 11% (males) 14% (females); 1000 ppm (week 104): ↑absolute weight 3% (females), ↑relative weight 10% (females)) Decrease in thymus weight at 1000 ppm in females (At week 52: ↓absolute weight 31%, ↓rel weight 23%; At week 104: ↓absolute weight 18%) Increase in pituitary weight noted in females at 1000 ppm (At week 52: ↑ rel weight 23%, ↑absolute weight 31%; At week 104: ↑ rel weight 38%, ↑absolute weight 32%) NOAEL: 50 ppm, equivalent to	

		6.4 mg/kg bw/day based on decreased (more than 20 %) cholinesterase activity in erythrocytes and brain (noted at ≥ 250 ppm, equivalent to 32.2 mg/kg bw/day) and organ weight changes noted at ≥250 ppm Tolclofos-methyl did not show any carcinogenic activity in this study.	

M: male

F: female

Table 53: Summary table of human data on STOT RE

No data

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

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
4-week (preliminary immunotoxicity study) Oral (dietary) OPPTS 870.7800 GLP: No *The study follows the guideline US OPPTS 870.7800, Immunotoxicity (Augusti 1998) except for following deviations:* *i. Water consumption was not determined (according to the guideline water consumption should be determined weekly)* *ii. A weekly physical examination was performed on each animal to monitor general health. The observations included were not specified in study report except for following categories : Coat, skin, build deformity.* *Study acceptable as supplementary data*	Tolclofos-methyl	Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. A weekly physical examination was performed on each animal to monitor general health. During the acclimatisation period, observations of the animals were recorded at least once per day. The weight of each mouse was recorded twice weekly before treatment started (day -7 and day -4), on the day of treatment start (day 1), twice weekly throughout the treatment period and before necropsy. The weight of each mouse in the positive control group was recorded on the first day of cyclophosphamide treatment. The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for the week before treatment start (week -1), and each week throughout the treatment period. At necropsy on day 29, blood samples and brains were obtained from all animals of groups. All animals were subject to a detailed necropsy. A full macroscopic examination of the samples of the following tissues were preserved in 10% neutral buffered formalin: lymph nodes, Payer´s patches and thymus. Samples of any abnormal tissues were also retained. The spleen from each animal was used as the sources of splenocytes. Splenocyte suspensions were prepared by mechanical dissociation and used for the plaque forming cell (PFC) assay. Duplicate tests per animal were evaluated. After preparation of the spleen cell suspensions, an assessment of cellular viability was performed during the cell counting stage using a Trypan blue dye exclusion method. The adaptive or acquired immune response of the animals was assessed using a modification of the Jerne Plaque Forming Cell assay (PFC assay). Animals were sensitised with a suspension of sheep red blood cells. This foreign antigenic preparation elicits a T-lymphocyte-dependent antibody response (TDAR) in the animals, which was measured by challenged leukocytes from the spleen in an ex vivo assay where sheep red blood cells are present in an agar matrix. This resulted in the formation of antibody-dependent lytic plaques, which were counted and indicated the activity of the immune response. All animals received an intravenous dose (by bolus injection) of	<u>100 ppm:</u> ↓plasma cholinesterase (31%) <u>2000 ppm:</u> ↓bw gain (52%) ↓plasma cholinesterase (86%) -changes in organ weights (↑rel. brain weight) <u>4500 ppm:</u> ↓bw gain (57%) ↓FC ↓plasma cholinesterase (89%) ↓brain cholinesterase (7%) -changes in organ weights (↑rel. brain weight)	RAR Vol. 3, B.6.8.1.2/01

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		sheep red blood cells (SRBCs) in 0.9% saline four days prior to necropsy. At necropsy the spleens from these animals were removed and a single cell suspension prepared from each animal. Viable cells were counted and diluted to an appropriate concentration for the assay (approximately 1 x 10^6 cells/mL and 2 x 10^6 cells/mL). The spleen cells were then mixed with an agar matrix containing a suspension of the SRBCs and guinea pig serum complement. This mixture was poured into petri dishes, allowed to set in incubated for up to 3 hours. Individual B lymphocytes secreting antibodies to SRBCs formed small foci where antibody-dependent complement mediated lysis occurs.		
Immunotoxicity study Oral (dietary) 4-week OPPTS 870.7800 GLP: Yes *The study follows the guideline US OPPTS 870.7800, Immuno-toxicity (Augusti 1998) except for following deviation:* *i. A weekly physical examination was performed on each animal to monitor general health. The observations included were not specified in study report except for following categories: skin colour, build deformity, coat.*	Tolclofos-methyl	Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. A weekly physical examination was performed on each animal to monitor general health. During the acclimatisation period, observations of the animals were recorded at least once per day. The weight of each mouse was recorded twice weekly before treatment started (day -7 and day -4), on the day of treatment start (day 1), twice weekly throughout the treatment period and before necropsy. The weight of each mouse in the positive control group was recorded on the first day of cyclophosphamide treatment. The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for the week before treatment start (week -1), and each week throughout the treatment period. For groups 1 to 4, water consumption was recorded the week before treatment start (week -1) and each week throughout the treatment period. Water consumption was recorded by weight for each cage. Animals were killed on day 29 by carbon dioxide asphyxiation followed by exsanguination. All animals were subjected to a detailed necropsy. A full macroscopic examination of the tissues was performed. The adrenals, spleen and thymus weights were recorded. The spleen from each animal was used as the sources of splenocytes. Splenocyte suspensions were prepared by mechanical dissociation and used for the plaque forming cell (PFC) assay. Duplicate tests per animal were evaluated. After preparation of the spleen cell suspensions, an assessment of cellular viability was performed during the cell counting stage	<u>500 ppm:</u> No treatment-related effects <u>1500 ppm:</u> No treatment-related effects <u>4500 ppm:</u> ↓bw gain (40%) ↓FC	RAR Vol. 3, B.6.8.1.2/02

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		using a Trypan blue dye exclusion method. The adaptive or acquired immune response of the animals was assessed using a modification of the Jerne Plaque Forming Cell assay (PFC assay). Animals were sensitised with a suspension of sheep red blood cells. This foreign antigenic preparation elicits a T-lymphocyte-dependent antibody response (TDAR) in the animals, which was measured by challenged leukocytes from the spleen in an ex vivo assay where sheep red blood cells are present in an agar matrix. This resulted in the formation of antibody-dependent lytic plaques, which were counted and indicated the activity of the immune response. All animals received an intravenous dose (by bolus injection) of sheep red blood cells (2×10^9 cells/mL) in 0.9% saline four days prior to necropsy. At necropsy the spleens from these animals were removed and a single cell suspension prepared from each animal. Viable cells were counted and diluted to an appropriate concentration for the assay (approximately 1×10^6 cells/mL and 2×10^6 cells/mL). The spleen cells were then mixed with an agar matrix containing a suspension of the SRBCs and guinea pig serum complement. This mixture was poured into petri dishes, allowed to set in incubated for up to 3 hours. Individual B lymphocytes secreting antibodies to SRBCs formed small foci where antibody-dependent complement mediated lysis occurs.		

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

In the 4-week toxicity study in the CD rats of Sprague Dawley strain, treatment was associated with clinical signs (patchy hair loss) noted in females at 20000 ppm (1830 mg/kg bw/day), reduced bodyweight gain noted in males (45%) and females (37%) at 20000 ppm (1830 and 1635 mg/kg bw/day), reduced food consumption noted in both sexes at 20000 ppm, changes in biochemical parameters noted in both sexes at 20000 ppm (higher cholesterol levels (both sexes), higher total protein and albumin values (males), higher inorganic phosphorus levels (females)), changes in organ weights noted at 1000 ppm (increased liver weights noted in both sexes at 20000 ppm and in females at 5000 ppm (414 mg/kg bw/day); increased kidney weights noted in males at ≥1000 ppm (79 mg/kg bw/day) and in females at 5000 ppm, and histopathological findings in the liver (hepatocyte enlargement) noted in both sexes at 20000 ppm. In addition, reduced cholinesterase activity was noted in all treated males, and in females at ≥5000 ppm. Lower plasma cholinesterase values were noted for both sexes at 20000 ppm (14% in males, 39% in females). Lower brain cholinesterase values were noted for males in all dose groups (reduced: 12%, 18%, 17% and 31%, for the 200, 1000, 5000, and 20000 ppm groups, respectively), and for females in the 5000 (20%) and 20000 ppm groups (21%). Lower erythrocyte cholinesterase values were noted for males in the 5000 ppm (18%) and 20000 ppm (19%) groups, and in females at 5000 ppm (19%). The NOAEL for male rats was set at

5000 ppm (corresponding to 414 mg/kg bw/day) based on reduced bodyweight gain (45%) noted at 20000 ppm, changes in clinical chemistry parameters (indicating liver toxicity) noted at 20000 ppm, reduced brain cholinesterase activity noted at 20000 ppm (31%), increased liver weights noted at 20000 ppm and histopathological findings in the liver (hepatocyte enlargement) noted at 20000 ppm. The NOAEL for female rats was set at 1000 ppm (corresponding to 88 mg/kg bw/day) based on reduced bodyweight gain (37%) noted at 20000 ppm, changes in clinical chemistry parameters (indicating liver toxicity) noted at 20000 ppm, reduced brain cholinesterase activity noted at 5000 ppm (20%) and 20000 ppm (21%), increased liver weights noted at 20000 ppm and histopathological findings in the liver (hepatocyte enlargement) noted at 20000 ppm (RAR Vol. 3, B.6.3.1.1/01).

In the 90-day toxicity study in the Crj:CD(SD) rat treatment was associated with clinical signs (loss of hair) noted in both sexes at 10000 ppm (653 and 696 mg/kg bw/day), reduced body weight noted in males (19%) and females (15%) at 10000 ppm, reduced bodyweight gain noted in males (25%) and females (27%) at 10000 ppm, reduced food consumption noted in both sexes at 10000 ppm, changes in haematological parameters noted in both sexes at 10000 ppm (males: increased activated partial thromboplastin time; females: decreased haemoglobin concentration (3%), decreased mean corpuscular haemoglobin (3%), decreased prothrombin time (2%), increased total leukocyte count, increased lymphocyte count), changes in biochemical parameters (indicating liver toxicity) noted in males at ≥100 ppm (≥6.46 mg/kg bw/day) and in females at ≥1000 ppm (≥71 mg/kg bw/day), changes in urinalysis parameters (decrease in pH) noted in both sexes at 10000 ppm, changes in organ weights noted at ≥1000 ppm (≥66.1 and ≥71 mg/kg bw/day in males and females, respectively) (At 1000 ppm: increased relative liver weight (males), increased relative kidney weight (females); At 10000 ppm: increased relative liver weight (both sexes), increased absolute liver weight (females), increased relative kidney weight (both sexes)), macroscopically changes in the liver (dark-red discoloration) noted in both sexes at 10000 ppm, and histopathological findings in the liver (hypertrophy) noted in both sexes at 10000 ppm. In addition, cholinesterase determination revealed decreases in plasma (males: 17%; females: 53%) and brain (males: 8%, females: 9%) cholinesterase levels in both sexes receiving 10000 ppm, and lower erythrocyte cholinesterase levels in both sexes (males: 17%; females: 20%) receiving 10000 ppm and in females receiving 1000 ppm (10%). The NOAEL for both sexes was set at 1000 ppm (corresponding to 66.1 and 71.0 mg/kg bw/day in males and females, respectively) based on reduced bodyweight and bodyweight gain noted in both sexes at 10000 ppm, changes in haematological and biochemical parameters noted in both sexes at 10000 ppm, reduced erythrocyte cholinesterase activities noted in females at 10000 ppm, increased liver and kidney weights noted in both sexes at 10000 ppm, macroscopically changes in the liver (dark-red discoloration) and histopathological findings in the liver (hepatocyte enlargement) noted in both sexes at 10000 ppm (RAR Vol. 3, B.6.3.1.1/02).

In the 6-month toxicity study in the Sprague Dawley rat, treatment was associated with reduced body weight noted in males (8%, not statistically significant) and females (15%) at 10000 ppm (540 and 623 mg/kg bw/day in males and females, respectively), reduced bodyweight gain noted in males (6%, not statistically significant) and females (18%) at 10000 ppm, changes in haematological parameters noted in females at 10000 ppm (reduced haemoglobin concentration (5%)), changes in biochemical parameters noted in females at ≥3000 ppm (≥184 mg/kg bw/day) (decreased uric acid concentration), changes in organ weights noted at ≥1000 ppm. At 1000 and 3000 ppm: increased absolute and relative liver weight (F), increased absolute and relative kidney weights (F); At 10000 ppm: increased relative liver weight (M, F), increased relative kidney weights (M, F), increased absolute kidney weight (F), increased testes weight). In addition, cholinesterase determination revealed decreases (23-40% during the first three months) in plasma in females at 10000 ppm, and erythrocyte cholinesterase levels were lowered in males at 3000 ppm (17%) and 10000 ppm (19%). The NOAEL for females was set at 300 ppm (corresponding to 18 mg/kg bw/day) based on reduced bodyweight (15%) and bodyweight gain (18%) noted at 10000 ppm, changes in haematological parameters noted at 10000 ppm (reduced haemoglobin concentration 5%), changes in biochemical parameters noted at ≥3000 ppm (decreased uric acid concentration), organ weight changes (increased kidney weights at ≥1000 ppm (65 mg/kg bw/day), increased liver weights at 10000 ppm) noted in females at 10000 ppm. The NOAEL for males was set at 3000 ppm (corresponding to 164 mg/kg bw/day) based on organ weight changes noted at 10000 ppm (RAR, Vol. 1, B.6.3.3.1/01).

Comment: The NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.3). It was agreed to set the NOAEL for kidney at 300 ppm (18 mg/kg bw per day). The kidney effect was considered as a critical effect in the study.

In the 9-month toxicity study in the mouse (ddY), treatment was associated with reduced bodyweight noted in males (21%) and females (23%) at 3000 ppm (513 and 564 mg/kg bw/day in males and females, respectively), reduced bodyweight gain noted in males (33%) and females (30%) at 3000 ppm, changes in biochemical parameters (increased cholesterol) noted in females at 3000 ppm and changes in organ weights (heart, testis, liver, kidney, brain, lung, adrenal, pituitary) noted at 3000 ppm. In addition, plasma cholinesterase activity was reduced in males at ≥100 ppm (≥12.2 mg/kg bw/day) and in females at ≥10 ppm (≥1.4 mg/kg bw/day), and erythrocyte cholinesterase activity was reduced in males and females at ≥100 ppm (≥12.2 and ≥13.8 mg/kg bw/day in males and females, respectively). Furthermore, brain cholinesterase activity was reduced in males at 3000 ppm. The NOAEL for males was set at 30 ppm (corresponding to 3.8 mg/kg bw/day) based on reduced body weight and bodyweight gain noted at 3000 ppm, changes in organ weights (liver, kidney, adrenal) noted at 3000 ppm, and decreased erythrocyte cholinesterase determination noted in erythrocytes at ≥100 ppm and in brain and plasma at 3000 ppm. The NOAEL for females was set at 100 ppm (corresponding to 13.8 mg/kg bw/day) based on reduced body weight and bodyweight gain noted at 3000 ppm, changes in organ weights (liver, kidney, adrenal) noted at 3000 ppm, and decreased cholinesterase determination in plasma and erythrocytes noted at 3000 ppm (RAR Vol. 3, B.6.3.4.1/01).

Comment: The NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.5). It was agreed to set the NOAEL for males at 30 ppm (corresponding to 3.8 mg/kg bw/day) based on decreased erythrocyte cholinesterase determination noted at ≥100 ppm.

In the 6-month toxicity study in the Beagle dog, treatment was associated with changes in haematological parameters noted in males at ≥600 ppm (≥24 mg/kg bw/day) (reduced haemoglobin and erythrocyte count) and in females at 2000 ppm (63 mg/kg bw/day) (reduced haemoglobin, haematocrit and erythrocyte count), changes in biochemical parameters (increased alkaline phosphatase) noted in both sexes at 2000 ppm (70 and 63 mg/kg bw/day in males and females, respectively), and changes in organ weights (increased absolute and relative liver weights) noted in both sexes at 2000 ppm. In addition, cholinesterase determination revealed decreases (19%) in plasma in females at 2000 ppm. The changes in haematological parameters (reduced haemoglobin and erythrocyte count) noted in males at 600 ppm were not considered adverse since they were transient, restricted to one sex and there were no coinciding effects observed at this dosage in all other parameters observed. The NOAEL for males was set at 600 ppm (corresponding to 24 mg/kg bw/day) based on changes in haematological parameters noted at 2000 ppm (reduced haemoglobin (>10%), reduced erythrocyte count, changes in biochemical parameters (increased alkaline phosphatase) and increased liver weights noted at 2000 ppm. The NOAEL for females was set at 600 ppm (corresponding to 21 mg/kg bw/day) based on changes in haematological parameters noted at 2000 ppm (reduced haemoglobin (>10%), haematocrit and erythrocyte count), changes in biochemical parameters noted at 2000 ppm (increased alkaline phosphatase), and organ weight changes (increased absolute and relative liver weights) noted at 2000 ppm (RAR, Vol. 3, B.6.3.3.2/01).

In the 1-year toxicity study in the Beagle dog, treatment was associated with reduced bodyweight noted in both sexes at 2000 ppm (59 and 62 mg/kg bw/day in males and females, respectively) (>10%, n.s), reduced bodyweight gain noted in both sexes at 2000 ppm (M: >10%, n.s; F: week 0-26: 65%, s.s.; week 0-52: 54%, n.s.), reduced food consumption noted in females at 2000 ppm, changes in haematological parameters noted in both sexes at 2000 ppm (decreased erythrocyte count, haematocrit, and haemoglobin values noted in both sexes, increased platelet count noted in males), changes in biochemical parameters noted in both sexes at 2000 ppm (decreased albumin values (s.s. in females only), decreases in total protein (s.s. in females only), elevated alkaline phosphatase values), changes in urinalysis noted in both sexes at 2000 ppm (increased incidence and severity of reducing substances), changes in organ weights noted in both sexes at 2000 ppm (increased mean absolute and relative weights for the liver, increased relative weights for the pancreas,

reduced absolute weight for the prostate), gross macroscopic findings noted in both sexes at 2000 ppm (liver: dark in colour; prostate: small in size), and histopathological findings in the liver noted in both sexes at ≥400 ppm (≥11.4 and ≥11.2 mg/kg bw/day in males and females, respectively) (At 400 ppm: increased incidence of hepatocytic pigment; At 2000 ppm: increased incidence of hepatocytic hypertrophy and intracytoplasmic homogeneous material and increased incidence of hepatocytic pigment). The NOAEL for male and females dogs was set at 400 ppm (corresponding to 11.4 and 11.2 mg/kg bw/day in males and females, respectively) based on reduced body weight growth noted in both sexes at 2000 ppm, changes in haematological parameters noted in both sexes at 2000 ppm (decreased erythrocyte count, haematocrit, and haemoglobin values noted in both sexes, increased platelet count noted in males), changes in biochemical parameters noted in both sexes at 2000 ppm (increased alkaline phosphatase in both sexes, decreased total protein in males (n.s.) and females (s.s.)), organ weight changes noted in both sexes at 2000 ppm (increased liver weights, increased pancreas weights, and reduced weight for prostate) and histopathological findings in the liver noted in both sexes at 2000 ppm (hepatocytic hypertrophy, intracytoplasmic homogenous material, hepatocytic pigment). The incidence of hepatocytic pigment noted at 400 ppm was not considered adverse since the grading was slight and no other treatment-related findings were noted in the liver at this dose level (RAR Vol. 3, B.6.3.5.1/01).

In the 21-day dermal toxicity study in the New Zealand White/Hazleton Dutchland rabbit, treatment was associated with dermal irritation noted in both sexes at ≥30 mg/kg bw/day, haematological changes (increased mean eosinophil value) noted in males at 1000 mg/kg bw/day, lower plasma cholinesterase activity noted in females at 300 and 1000 mg/kg bw/day, organ weight changes (increased relative kidney) noted in females at 1000 mg/kg bw/day, and histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration) noted in both sexes at ≥30 mg/kg bw/day. The NOAELsystemic for male rabbits was set at ≥1000 mg/kg bw/day. The NOAEL systemic for female rabbits was set at 300 mg/kg bw/day based on increased (20%) relative kidney weight noted at 1000 mg/kg bw/day. No NOAEL was set for local effects since dermal irritation was noted in all treated groups (RAR Vol. 3, B.6.3.6/01).

Comment: The NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.4). It was agreed to set the systemic NOAEL for female rabbits at 300 mg/kg bw per day based on increased (20%) relative kidney weight noted at 1000 mg/kg bw/day.

In the subchronic neurotoxicity study in the Alpk:AP_fSD (Wistar-derived) rat, oral administration of 10000 ppm (735.7 and 762.7 mg/kg bw/day in males and females, respectively) tolclofos-methyl for at least 90 days resulted in reductions in body weights (males: 17%, females: 11%), bodyweight gains (males: 31%, females: 18%) and food consumption. In addition, erythrocyte cholinesterase activity was lowered in males at ≥1800 ppm (≥122.3 mg/kg bw/day) and in females at ≥300 ppm (≥23.1 mg/kg bw/day). Furthermore, brain cholinesterase activity was lowered in males at 10000 ppm and in females at ≥1800 ppm (≥143.9 mg/kg bw/day). There were no neuropathology findings at dose levels up to and including 10000 ppm tolclofos-methyl. The NOAEL was 1800 ppm tolclofos-methyl (122.3 mg/kg bw/d in males and 135.8 mg/kg bw/day in females) based on reduced body weight and bodyweight gain noted in both sexes at 10000 ppm, and lowered cholinesterase activity noted in males at 10000 ppm (reduced erythrocyte cholinesterase: 25%, reduced brain cholinesterase: 14%) (RAR Vol. 3, B.6.7.1.2/01).

In a three generation reproduction study in Strague Dawley, dose levels up to 1000 ppm (70.6 mg/kg bw/day) were used. No systemic toxic effects were noted in the parental and in the pup generations nor a reprotoxic effect was observed. The parental NOAEL was ≥1000 ppm (≥70.6 mg/kg bw/day). The NOAEL for pups and reproduction were ≥1000 ppm (≥70.6 mg/kg bw/day). The dose levels used in the study were low (RAR Vol. 3 B.6.6.1/01).

In the one generation reproductive study in Crj:CD(SD)(SPF) rats, dose level up to 10000 ppm (680 and 668 mg/kg bw/day in males and females, respectively) were used. Systemic toxicity for both the parental and the offspring generation comprised a reduction in body weights, bodyweight gains and food consumption, and increased liver and kidney weights noted in the parental animals at ≥5000 ppm (≥338 mg/kg bw/day) and in the offspring at ≥2500 ppm (≥255 mg/kg bw/day). Furthermore, the offspring generation showed an increased prostate weight (relative weight increased 25%) at 10000 ppm (1161 mg/kg bw/day). In addition to these findings, a decrease in brain cholinesterase activity (less than 20%) was observed in the parental animals at ≥5000 ppm. The reproductive performance was not affected by tolclofos-methyl treatment. A delay of starting and completing separation was noted in the offspring at 10000 ppm. This delay was considered a secondary effect due to the body weight suppression noted at this dose level. The histopathological examination of genital organs revealed no treatment related changes. For the parental generation, the NOAEL for systemic toxicity was 2500 ppm (173 and 178 mg/kg bw/day in males and females, respectively) based on reduced bodyweight gain noted in females at ≥5000 ppm and in males at 10000 ppm, reduced body weight noted in females at 10000 ppm, and organ weight changes in the liver and kidneys noted at ≥5000 ppm. NOAEL for the offspring generation could not be derived. Reduced body weights and bodyweight gains and increased organ weights of liver and kidney were noted in all treated groups. In addition, the relative weight of prostate was increased (25%) at 10000 ppm. NOAEL for reproductive toxicity was set at ≥10000 ppm (≥680 and ≥668 mg/kg bw/day in males and females, respectively) (RAR Vol. 3, B.6.6.1/02).

In a developmental toxicity study in the rat, dose levels up to 50 mg/kg bw/day were tested. No adverse maternal toxicity or embryo-toxic, or teratogenic effects were noted in the study. NOAEL was set at ≥50 mg/kg bw/day. The dose levels used in the study were low (RAR, Vol. 3, B.6.6.2.1/01).

In another developmental toxicity study in the rat, dose levels up to 1000 mg/kg bw/day were tested. Treatment was associated with reduced maternal bodyweight gain (Days 6-11: 27%, net bodyweight change of 14% showed a statistically significant negative trend) noted in dams at 1000 mg/kg bw/day. Delayed ossification of the 5th and/or 6th sterneabrae was seen in the fetuses at 1000 mg/kg bw/day. The NOAEL for maternal toxicity was set at 300 mg/kg bw/day. NOAEL for developmental toxicity was set at 300 mg/kg bw/day based on increased incidence of non-ossified sternebrae noted at 1000 mg/kg bw/day (RAR, Vol. 3, B.6.6.2.1/02).

Comment: The NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.7). The maternal and developmental NOAEL was concluded to be set at 300 mg/kg bw/day.

In a developmental toxicity study in the rabbit, treatment was associated with clinical signs (abortions) noted at ≥1000 mg/kg bw/day and mortality (one dam) noted at 3000 mg/kg bw/day. Furthermore, reduced bodyweights were noted in dams at ≥300 mg/kg bw/day and reduced bodyweight gain were noted in dams at ≥1000 mg/kg bw/day. In addition, organ weight changes were noted in dams at ≥1000 mg/kg bw/day (At 1000 mg/kg bw/day: decreased kidney weight; At 3000 mg/kg bw/day: decreased kidney and spleen weights). Embryofetal development was not impaired. NOAEL for maternal toxicity was set at 300 mg/kg bw/d based on abortions noted at ≥1000 mg/kg bw/day, mortality noted in one dam at 3000 mg/kg bw/day, reduced body weight noted at ≥1000 mg/kg bw/day and reduced bodyweight gain noted at ≥1000 mg/kg bw/day. NOAEL for developmental toxicity was set at 300 mg/kg bw/day based on abortions noted at ≥1000 mg/kg bw/day (RAR, Vol. 3, B.6.6.2.1/03).

Comment: Maternal and developmental NOAEL in the study was discussed at Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.8). The maternal NOAEL was agreed to be set at 300 mg/kg bw/day based on reduced bodyweight gain and abortion. Abortion was also considered the critical effect for developmental toxicity and the NOAEL for developmental was set at 300 mg/kg bw/day.

In the combined chronic/carcinogenic toxicity test in the rat, no signs of compound effect were observed with regard to mortality, clinical signs, body weights, food consumption, organ weights and organ/body weight ratios, and gross pathology. No histomorphological alterations were attributable to the test compound at dietary levels up to 1000 ppm (42 and 49 mg/kg bw/day in males and females, respectively). Tolclofos-methyl was not oncogenic in this study. The study is acceptable, however, it can be noted that the high dose, 1000 ppm, represent a lower dose in mg/kg bw from week 52 until termination than what was established to be the minimum toxic level in the subacute and subchronic toxicity studies. It is therefore not possible to set the precise NOAEL for the study; NOAEL: ≥ 1000 ppm (42 mg/kg bw/day) (RAR Vol. 3, B.6.5.1/01)

The assessment of cholinesterase activity was assessed in a second long-term toxicity study in the rat. In this study samples were taken from 10 animals/sex/group at initiation of the study, at weeks 5, 14, 27, 53, 79 and at termination for blood cholinesterase determination; for brain cholinesterase activity determination, samples were taken at week 53 and at termination. Food consumption and body weights were measured weekly during the first 26 weeks, once every two weeks from weeks 26 through 52 and once every four weeks from weeks 53 through 104. Examination and palpation for incidence and location of tissue masses were performed at each weighing interval. All animals were subjected to gross pathology at necropsy. There were no compound related effect observed with regard to mortality, clinical signs, body weights, food consumption and gross pathology. Mean erythrocyte cholinesterase and brain cholinesterase activities were comparable among groups throughout the study. NOAEL: ≥1000 ppm (equivalent to 42 mg/kg bw/day). The study is regarded as supplementary data only (RAR Vol. 3, B.6.5.1/02)

In the long-term toxicity study in the mouse, treatment with tolclofos-methyl was associated with reduced bodyweight gain after 52 weeks in the female 1000 ppm group (137 mg/kg bw/day) (15%) and decreased food consumption at week 104 in the female 1000 group. Decrease in plasma, erythrocyte and brain cholinesterase was noted in both sexes at 250 (32.2 and 34.1 mg/kg bw/day in males and females, respectively) and 1000 ppm (134 and 137 mg/kg bw/day in males and females, respectively). At week 28 in females, the cholinesterase activity was decreased already at 50 ppm (6.9 mg/kg bw/day) (reduction in plasma (12%) and brain (18%)). At week 28 (250 ppm group animals), the erythrocyte cholinesterase activity was reduced at a magnitude of 26% in males and 28% in females, and the magnitude of reduced plasma cholinesterase activity was 41% (250 ppm group males) and 52% (250 ppm group females). Furthermore the brain cholinesterase activity at the same dose level and time point was reduced at a magnitude of 24% in females. At week 104 (250 ppm group animals), the plasma cholinesterase activity was reduced 25% in males and 33% in females; erythrocyte cholinesterase activity was reduced 13% in males and 11% in females and the brain cholinesterase activity reduced 13% in males. At week 104 (1000 ppm) the plasma cholinesterase activity was reduced 43% in males and 59% in females, the erythrocyte cholinesterase activity was reduced 13% in males and 23% in females and the brain cholinesterase activity was reduced 26% in males and 9% in females. At week 52 (250 ppm) the erythrocyte cholinesterase activity was reduced 23% in females and 18% in males, and brain cholinesterase activity was reduced 12% in females and 14% in males. Furthermore increased glucose was noted in male 1000 ppm group after 104 weeks. Organ weight changes were noted in both sexes at 250 ppm and above. Increase in kidney weights were noted in both sexes at ≥250 ppm, reduced thymus weight was noted at 1000 ppm in females and increased pituitary weight was noted in females at 1000 ppm. Tolclofos-methyl did not show any carcinogenic activity in this study (RAR Vol. 3, B.6.5.2/01).

In a preliminary 4-week immunotoxicity study in female CD-1 mice, dietary administration of tolclofos-methyl at concentrations up to 4500 ppm (749 mg/kg bw/day) caused a non-specific toxic response at 2000 ppm (413 mg/kg bw/day) or more and a small suppression of brain cholinesterase activity at 4500 ppm. In addition plasma cholinesterase activity was reduced at ≥100 ppm (≥19.6 mg/kg bw/day). There was no effect on the immune function. The NOAEL for immunotoxicity to tolclofos-methyl was greater than 4500 ppm in females (equaling 749 mg/kg bw/day).

In a 4-week immunotoxicity study in female CD-1 mice, dietary administration of tolclofos-methyl at concentrations up to 4500 ppm (811 mg/kg bw/day) caused a non-specific toxic response at 4500 ppm based on decreased bodyweight gain and transient reduction of food consumption. No effect on the immune function was observed. The NOAEL for immuno-toxicity was established to exceed 4500 ppm in females (equaling 811 mg/kg bw/day).

Table 55: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Not considered adequate.

10.12.2 Comparison with the CLP criteria

Regulation EC No 1272/2008 (CLP), Annex 1: 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 degeneration and reduced cell number) in vital organs incapable of regeneration.

According to the CLP Guidance Table 3.9.3, a substance should be classified in Category 2 when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in table below:

Route of Exposure	Units	Guidance Values Ranges: (dose/concentration)
Oral (rat)	mg/kg bw/day	10<C≤100
Dermal (rat or rabbit)	mg/kg bw/day	20<C≤200
Inhalation (rat) gas	ppm V/6h/day	50<C≤250
Inhalation (rat) vapour	mg/litre/6h/day	0.2<C≤1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	0.02<C≤0.2

According to Annex 1 3.9.2.9.8, the guidance values in table above is increased by a factor of three for a 28-day study.

4-week feeding study in rats (RAR Vol. 3, B.6.3.1.1/01)

In this study, rats (Sprague Dawley) were administered tolclofos-methyl in the diet for 4-weeks at doses up to 20000 ppm (1635 and 1830 mg/kg bw/day in males and females, respectively). The liver and the kidneys were the apparent target organs in the study. Effects on the liver consisted of increased organ weights, changes in biochemical parameters, and histopathological changes (hepatocyte enlargement) noted in both sexes at 20000 ppm (1635 and 1830 mg/kg bw/day in males and females, respectively). The findings in the kidneys consisted of increased organ weights noted in males from 1000 ppm (79 mg/kg bw/day) and in females from 5000 ppm (452 mg/kg bw/day). The findings in the liver and kidneys were not considered severe enough for a STOT-RE classification. Furthermore, reduced brain cholinesterase activity was noted in males at 200 ppm (16 mg/kg bw/day) (12%), 1000 ppm (79 mg/kg bw/day) (18%), 5000 ppm (414 mg/kg bw/day) (17%) and 20000 ppm (1635 mg/kg bw/day (31%), and in females at 5000 ppm (452 mg/kg bw/day) (20%) and 20000 ppm (1830 mg/kg bw/day) (21%). Reduced erythrocyte cholinesterase activity was noted for males at 200 ppm (16 mg/kg bw/day) (10%), 5000 ppm (414 mg/kg bw/day) (18%) and 20000 ppm (1635 mg/kg bw/day) (19%), and in females at 5000 ppm (452 mg/kg bw/day) (19%). Reduced plasma cholinesterase was noted in males (14%) and females (39%) at 20000 ppm (1635 and 1830 mg/kg bw/day in males and females, respectively). The magnitude of reduced cholinesterase activity was considered adverse at the dose level of ≥5000 ppm (414 and 452 mg/kg bw/day in males and females, respectively). The effects noted in the study were not considered of concern for a classification as STOT-RE No adverse effects were noted within the critical range of doses for Cat 2 classification (i.e. $30 < C \leq 300$ mg/kg bw/day) (Haber´s rule considered).

90-day feeding study in rats (RAR Vol. 3, B.6.3.1.1/02)

In this study, rats (CD(SD)) were administered tolclofos-methyl in the diet for 90-days at doses up to 10000 ppm (653 and 696 mg/kg bw/day in males and females, respectively). The liver and the kidneys were the apparent target organs in the study. Effects on the liver consisted of increased organ weights noted in males at 1000 ppm (66.1 mg/kg bw/day) and 10000 ppm (653 mg/kg bw/day), and in females at 10000 ppm (696 mg/kg bw/day). Changes in biochemical parameters (indicating liver toxicity) were noted in males from 100 ppm (6.46 mg/kg bw/day) and in females from 1000 ppm (66.1 mg/kg bw/day). Furthermore, histopathological changes in the liver (hypertrophy) were noted in both sexes at 10000 ppm (653 and 696 mg/kg bw/day in males and females, respectively). Effects on the kidneys consisted of increased organ weights noted in females from 1000 ppm (71 mg/kg bw/day) and in males at 10000 ppm (653 mg/kg bw/day). The findings in the liver and kidneys were not considered severe enough for a STOT-RE classification. Reduced haemoglobin concentration (3%) was noted in females at 10000 ppm (696 mg/kg bw/day). Furthermore, reduced glucose was noted in females from 1000 ppm (71 mg/kg bw/day) but this effect was not considered critical for a STOT-RE classification. Reduced erythrocyte cholinesterase values (17% in males, 20% in females) were noted in both sexes at 10000 ppm (653 and 696 mg/kg bw/day in males and females, respectively). Reduced plasma cholinesterase activities (17% in males, 53% in females) were also noted in both sexes at 10000 ppm (653 and 696 mg/kg bw/day in males and females, respectively). Furthermore the brain cholinesterase activities were reduced in males (8%) and in females (9%) at 10000 ppm (653 and 696 mg/kg bw/day in males and females, respectively). The effects noted in the study were not considered of concern for a classification as STOT-RE since no adverse effects were noted within the critical range of doses for Cat 2 classification (i.e. $10 < C \leq 100$ mg/kg bw/day).

6-months feeding study in rats (RAR Vol. 3, B.6.3.3.1/01)

In this study rats (Sprague Dawley) were administered tolclofos-methyl in the diet for 6 months at doses up to 10000 ppm (540 and 623 mg/kg bw/day in males and females, respectively). The liver and the kidneys were the apparent target organs in the study. Effects on the liver and the kidneys consisted of increased organ weights noted in females at ≥1000 ppm (65 mg/kg bw/day) and in males at 10000 ppm (540 mg/kg bw/day). The findings in the liver and kidneys were not considered severe enough for a STOT-RE classification. Reduced erythrocyte cholinesterase values were noted in males at 3000 ppm (164 mg/kg bw/day) (17%) and

1000 ppm (540 mg/kg bw/day) (19%), and reduced plasma cholinesterase activity was noted in females during the first 3 months at 10000 ppm (623 mg/kg bw/day) (23-40%). Furthermore, reduced haemoglobin concentration (5%) was noted in females at 10000 ppm (623 mg/kg bw/day). The effects noted in the study were not considered of concern for a classification as STOT-RE since no adverse effects were noted within the critical range of doses for Cat 2 classification (i.e. $5 < C \leq 50$ mg/kg bw/day) (Haber´s rule considered).

9-month mouse (RAR Vol. 3, B.6.3.4.1/01)

In this study mice (ddY) were administered tolclofos-methyl in the diet for 9 months at doses up to 3000 ppm (513 and 564 mg/kg bw/day in males and females, respectively). The liver, kidneys and adrenals were the apparent target organs in the study. Effects on the liver consisted of increased cholesterol concentration (females only) and increased organ weights (both sexes) noted at 3000 ppm (513 and 564 mg/kg bw/day in males and females, respectively). At the same dose level relative adrenal weights were increased in both sexes. The findings in the liver, kidneys and adrenals were not considered severe enough for a STOT-RE classification. Reduced erythrocyte cholinesterase activities were noted in males at 100 ppm (12.2 mg/kg bw/day) (20%) and 3000 ppm (513 mg/kg bw/day) (55%), and also in females at 100 ppm (13.8 mg/kg bw/day) (13%) and 3000 ppm (35%). Reduced plasma cholinesterase activities were noted in males at 100 ppm (12.2 mg/kg bw/day) (44%) and 3000 ppm (513 mg/kg bw) (95%), and also in females at 100 ppm (13.8 mg/kg bw/day) (58%) and 3000 ppm (564 mg/kg bw/day) (88%). Furthermore, reduced brain cholinesterase activity was noted in males at 3000 ppm (513 mg/kg bw/day) (25%). The effects on cholinesterase activity (reduction in erythrocyte cholinesterase activity (20%) and plasma cholinesterase activity (44%)) noted in males at 100 ppm (12.2 mg/kg bw/day) were noted at a dose within the critical range of doses for Cat 2 classification (i.e. $3 < C \leq 33$ mg/kg bw/day) (Haber´s rule considered). However, no clinical signs were noted in the study. Therefore the effect was not considered severe enough for STOT-RE classification by the RMS.

6-month feeding study in dogs (RAR Vol. 3, B.6.3.3.2/01):

In this study dogs (Beagle) were administered tolclofos-methyl in the diet for 6 months at doses up to 2000 ppm (70 and 63 mg/kg bw/day in males and females, respectively). The liver was the apparent target organ. Effects on the liver consisted of increased alkaline phosphatase and increased organ weights noted in both sexes at 2000 ppm (70 and 63 mg/kg bw/day in males and females, respectively). The findings in the liver were not considered severe enough for a STOT-RE classification. Reduced haemoglobin was noted in males at 600 ppm (24 mg/kg bw/day) (11% at week 12; 8% at week 16) and 2000 ppm (70 mg/kg bw/day) (14% at week 12; 13% at week 24), and in females at 2000 ppm (63 mg/kg bw/day) (11% at week 20, 14% at week 23). The magnitude of this effect (<20%) was not considered adverse for a STOT-classification. Furthermore, reduced plasma cholinesterase activity was noted in females at 2000 ppm (63 mg/kg bw/day) but the magnitude (19%) of this effects was not considered adverse, and no effects were noted on erythrocyte cholinesterase activity. The effects noted in the study were not considered of concern for a classification as STOT-RE since no severe effects occurred within the critical range of doses for Cat 2 classification (i.e. $5 < C \leq 50$ mg/kg bw/day) (Haber´s rule considered).

1-year feeding study in dogs (RAR Vol. 3, B.6.3.5.1/01):

In this study, dogs (Beagle) were administered tolclofos-methyl in the diet for 1-year at doses up to 2000 ppm (59 and 62 mg/kg bw/day in males and females, respectively). The liver, pancreas and prostate were the apparent target organs. Effects on the liver consisted of increased alkaline phosphatase, increased organ weights, and histopathological changes (hepatocytic hypertrophy, intracytoplasmic homogenous material and hepatocytic pigment) noted in both sexes at 2000 ppm (59 and 62 mg/kg bw/day in males and females, respectively). Slight hepatocytic pigment was also noted in both sexes at 400 ppm (11.4 and 11.2 mg/kg bw/day in males and females, respectively) but was not considered adverse in the absence of other effects noted at this dose level. Effects on the pancreas and prostate consisted of organ weight changes (pancreas: increased relative weight; prostate: reduced absolute weight) noted at 2000 ppm (59 and 62 mg/kg bw/day in

males and females, respectively). The findings noted in the liver, pancreas and prostate were not considered severe enough for a STOT-RE classification. Changes in urinalysis (increased incidence and severity of reducing substances) were noted in both sexes at 2000 ppm (59 and 62 mg/kg bw/day in males and females, respectively). Furthermore, reduced haemoglobin was noted in males (17% s.s.) and in females (8% n.s.) at 2000 ppm (59 and 62 mg/kg bw/day in males and females, respectively). The effects noted in the study were not considered of concern for a classification as STOT-RE since no adverse effects occurred within the critical range of doses for Cat 2 classification (i.e. $2.5 < C \leq 25$ mg/kg bw/day) (Haber´s rule considered).

21-day dermal toxicity study in rabbits (RAR Vol. 3, B.6.3.6/01):

In this study rabbits (New Zealand White) were administered tolclofos-methyl by the dermal route for 21-days at 1000 mg/kg bw/day. The kidney was the apparent target organ. Effects on the kidney consisted of increased organ weights noted in females at 1000 mg/kg bw/day. Furthermore, reduced plasma cholinesterase activities were noted in females at 300 mg/kg bw/day (29%) and 1000 mg/kg bw/day (25%) but this effect was not considered adverse in the absence of effects on cholinesterase activity in the brain or erythrocytes. The effects noted in the study were not considered of concern for a classification as STOT-RE. No adverse effects were noted within the critical range of doses for Cat 2 classification (i.e. $60 < C \leq 600$ mg/kg bw/day).

Subchronic neurotoxicity study in rats (RAR Vol. 3, B.6.7.1.2/01):

In this study Alpk:APf(SD) rats were administered tolclofos-methyl in the diet for 90 days at doses up to 10000 ppm (735.7 and 762.7 mg/kg bw/day in males and females, respectively). In the satellite group reduced erythrocyte cholinesterase activity was noted in males at 1800 ppm (130.2 mg/kg bw/day) (10%) and 10000 ppm (719.7 mg/kg bw/day) (18%), and in females at 300 ppm (24.3 mg/kg bw/day) (8%), 1800 ppm (143.9 mg/kg bw/day) (14%) and 10000 ppm (817.5 mg/kg bw/day) (15%). Furthermore, brain cholinesterase activity was reduced in males at 10000 ppm (817.5 mg/kg bw/day) (14% at week 5), and in females at 1800 ppm (143.9 mg/kg bw/day) (13% at week 14) and 10000 ppm (817.5 mg/kg bw/day) (14% at week 14). The effects noted in the study were not considered of concern for a classification as STOT-RE since no adverse effects were noted within the critical range of doses for Cat 2 classification (i.e. $10 < C \leq 100$ mg/kg bw/day). The magnitude of reduced cholinesterase activity was less than 20% at doses within the critical range of doses for Cat 2 classification.

Rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/01):

Reduced mean implantation efficiency (86.1% compared to 91.9% in control group) were noted at 50 mg/kg bw/day. In the absence of other effects, this effect was not considered adverse. Thus no concern for classification was observed in this study.

Rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/02):

Maternal findings were noted at 1000 mg/kg bw/day and consisted of reduced bodyweight gain (27%, net bodyweight change of 14% showed a statistically significant negative trend). Delayed ossification of sterneabrae was seen in the fetuses at 1000 mg/kg bw/day. The incidence of delayed ossification of sterneabrae occurred at maternal toxic dose level. In the absence of other effects this effect was not considered of concern for classification with STOT-RE.

Rabbit developmental toxicity study (RAR Vol. 3, B.6.6.2.1/03)

Adverse maternal findings were noted at ≥1000 mg/kg bw/day. These findings consisted of mortality (one dam at 3000 mg/kg bw/day), abortions (noted at ≥1000 mg/kg bw/day), reduced bodyweight and bodyweight

gain (noted at ≥1000 mg/kg bw/day). No treatment-related effects were noted in the pups except for abortions. The effect on abortion observed in rabbit developmental study was considered suitable for the setting of adversity but not enough for classification as the incidence of abortion was very low and observed at the limit dose of the OECD guideline (1000 mg/kg bw per day). Thus no concern for classification was observed in this study.

Three-generation reproductive toxicity study (RAR Vol. 3, B.6.6.1/01):

In this study Strague Dawley rats were administered tolclofos-methyl in the diet at dose levels up to 1000 ppm (70.6 mg/kg bw/day). No systemic toxic effects were noted in the parental and in the pup generations, nor a reprotoxic effect was observed. Thus no concern for classification was observed in this study.

One generation reproductive toxicity study (RAR Vol. 3, B.6.6.1/02):

In this study Crj:CD(SD)(SPF) rat were administered tolclofos-methyl in the diet at dose level up to 10000 ppm (680 and 668 mg/kg bw/day in males and females, respectively). Systemic toxicity for both the parental and the offspring generation comprised a reduction in body weights, bodyweight gains and food consumption, and increased liver and kidney weights noted in the parental animals at ≥5000 ppm (≥338 mg/kg bw/day) and in the offspring at ≥2500 ppm (≥255 mg/kg bw/day). Furthermore, the offspring generation showed an increased prostate weight (relative weight increased 25%) at 10000 ppm (1161 mg/kg bw/day). In addition to these findings, a decrease in brain cholinesterase activity (less than 20%) was observed in the parental animals at ≥5000 ppm. The reproductive performance was not affected by tolclofos-methyl treatment. A delay of starting and completing separation was noted in the offspring at 10000 ppm. This delay was considered a secondary effect due to the body weight suppression noted at this dose level. The histopathological examination of genital organs revealed no treatment related changes. The effects noted were not considered severe enough for STOT-RE classification.

Combined two year chronic toxicity/carcinogenicity study in rats (RAR Vol. 3, B.6.5.1/01)

In this study rats (Fischer 344 CD®F) were administered tolclofos-methyl orally via the diet for 2 years at doses up to 1000 ppm (42 and 49 mg/kg bw/day in males and females, respectively). No distinct signs of compound effect were observed with regard to mortality, clinical signs, body weights, food consumption, organ weights and organ/body weight ratios, gross pathology and histopathology. Minor changes in clinical chemistry parameters (↓alkaline phosphatase) were noted in males of all treated groups. This effects was not considered severe for STOT-RE classification.

2-year chronic toxicity study in rats (RAR Vol. 3, B.6.5.1/02):

In this study Fischer 344 rats were administered tolclofos-methyl orally via the diet for 104 weeks at doses up to 1000 ppm (42 and 49 mg/kg bw/day in males and females, respectively). There were no compound related effects observed with regard to mortality, clinical signs, body weights, food consumption and gross pathology. Mean erythrocyte cholinesterase and brain cholinesterase activities were comparable among groups throughout the study. Thus no concern for STOT-RE was observed in this study.

24 month toxicity study in mice (RAR Vol. 3, B.6.5.2/01)

In this study Crj:B6C3F1 mice were administered tolclofos-methyl orally via the diet for 24 months at doses up to 1000 ppm (134 and 137 mg/kg bw/day in males and females, respectively). Organ weight changes were noted in brain, pituitary, kidneys and liver from 250 ppm (32.2 and 34.1 mg/kg bw/day in males and females, respectively) and in thymus (females) at 1000 ppm (137 mg/kg bw/day). Increased glucose concentrations were noted for males at 1000 ppm (134 mg/kg bw/day). These effects on organ weights and glucose concentrations were not considered critical for STOT-RE classification. Reduced cholinesterase activity were noted from 250 ppm (32.2 and 34.1 mg/kg bw/day in males and females, respectively). At week 28

(250 ppm group animals), the erythrocyte cholinesterase activity was reduced at a magnitude of 26% in males and 28% in females, and the magnitude of reduced plasma cholinesterase activity was 41% (250 ppm group males) and 52% (250 ppm group females). Furthermore the brain cholinesterase activity at the same dose level and time point was reduced at a magnitude of 24% in females. The effects on cholinesterase activity (reduction >20%) during week 28 at the dose level of 250 ppm (32.2 and 34.1 mg/kg bw/day in males and females, respectively) were noted at a dose within the critical range of doses for Cat 2 classification (i.e. $5 < C \leq 50$ mg/kg bw/day) (Haber´s rule considered for exposure durations of 28 weeks). However, no clinical signs were noted in the study. Therefore the effect was not considered severe enough for STOT-RE classification by the RMS.

Immunotoxicity study (RAR Vol. 3, B.6.8.1.2/01):

In this study female mice (Crl:CD1(1CR)) were administered tolclofos-methyl in the diet for four weeks at doses up to 4500 ppm (811 mg/kg bw/day). No specific effects were observed in the study except for decreased bodyweight gain and transient reduction of food consumption. These effects noted in the study were not considered of concern for classification as STOT-RE.

Preliminary 4-week immunotoxicity study (RAR Vol. 3, B.6.8.1.2/01):

In this study female mice (Crl:CD1(1CR)) were administered tolclofos-methyl in the diet for four weeks at doses up to 4500 ppm (749 mg/kg bw/day). Reduced bodyweight gain were noted at 2000 (413 mg/kg bw/day) and 4500 ppm. Reduced cholinesterase levels were noted in brain at 4500 ppm (7%). The magnitude of inhibition was not considered adverse. There were no effects on erythrocyte cholinesterase activity in the study. The effects noted in the study were not considered of concern for classification as STOT-RE.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification is proposed for tolclofos-methyl by RMS

Comment: The need of a classification with STOT RE Cat 2 was a discussion point at the Pesticides Peer Review (PRR) Meeting 162, September 2017 (Evaluation table Expert´s consultation 2.2). However, EFSA proposed no further discuss this point since it is not critical for risk assessment and approval criteria. No conclusion by the pesticide peer review on this point.

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

Summary of the Dossier Submitter's proposal

Six oral repeated dose toxicity studies were available; three in rats (for 28 days, 90 days, and 6 months), one in mice (for 9 months) and two in dogs (for 6 months and one year). Additionally, combined two year chronic toxicity/carcinogenicity studies were available (one in rats and one in mice), as well as a 90-day neurotoxicity study in rats, a 104-week cholinesterase activity study in rats, and two 4-week immunotoxicity studies in rats. For the dermal route, one 21-day dermal repeated dose toxicity study was available in rabbits.

The table below presents the effects in these studies at relevant doses for classification.

***Table**: Summary of repeated dose toxicity studies with tolclofos-methyl*

Study	**Dose levels**	**Target organ(s)** **NOAEL**	**Effects at relevant doses for classification**
ORAL			
4-week (diet) Rat, CD of Sprague Dawley strain (10/sex/dose) In-house method, in accordance with 92/69/EEC, B.7 GLP (Anon. 1982; RAR Vol.3 B.6.3.1.1/01)	0, **200**, **1000**, 5000, 20000 ppm equal to m: 0, 16, 79, 414, 1635 mg/kg bw/d f: 0, 18, 88, 452, 1830 mg/kg bw/d Guidance value for classification **≤ 300** mg/kg bw/d	Liver, kidney, nervous system NOAEL m: 5000 ppm NOAEL f: 1000 ppm	200 ppm: ↓brain cholinesterase activity (m: 12%) ↓erythrocyte cholinesterase activity (m: 10%, f: 11%) 1000 ppm: ↓brain cholinesterase activity (m: 18%) - organ weight changes (↑kidney (m) rel: 14%, abs: 9%)
90-day (diet) Rat, Crj:CD(SD) (12/sex/dose) FIFRA §82-1 GLP (Anon. 1990, RAR Vol.3 B.6.3.2.1/01)	0, **100**, **1000** and 10000 ppm equal to m: 0, 6.46, 66.1, 653 mg/kg bw/d f: 0, 7.13, 71.0, 696 mg/kg bw/d Guidance value for classification **≤ 100** mg/kg bw/d	Liver, kidney, nervous system NOAEL m/f: 1000 ppm	100 ppm: - changes in biochemical parameters (↓glutamic-oxaloacetictransaminase (m: 14%)) 1000 ppm: - changes in biochemical parameters (↓glutamic-oxaloacetictransaminase (m: 17%), ↑α2-globulin (m: 13%), ↑inorganic phosphorus (f: 14%), ↓γ-globulin (f: 14%), ↓glucose (f: 11%) ↓erythrocyte cholinesterase activity (f:10%)) - organ weight changes (↑rel liver (m: 4%), ↑rel kidney (f: 10%))
6-month (diet) Rat, Sprague Dawley (15/sex/dose) In-house method No GLP but a QA statement (Anon. 1978, RAR Vol.3 B.6.3.3.1/01)	0, **300**, 1000, 3000 and 10000 ppm equal to m: 0, 16, 51, 164, 540 mg/kg bw/d f: 0, 18, 65, 184, 623 mg/kg bw/d Guidance value for classification **≤ 50** mg/kg bw/d	Liver, kidney, nervous system NOAEL m: 3000 ppm NOAEL f: 300 ppm	300 ppm: None 1000 ppm*: - changes in organ weights (↑ liver (f) abs 10%, rel 8%), ↑ kidney (f) abs 25%, rel 24%))
9-month (diet) Mouse, ddY (15/sex/dose) EPA Guideline 82-1 No GLP (Anon. 1978, RAR Vol.3 B.6.3.4.1/01)	0, **10, 30, 100** and 3000 ppm equal to m: 0, 1.2, 3.8, 12.2, 513 mg/kg bw/d f: 0, 1.4, 4.1, 13.8, 564 mg/kg bw/d Guidance value for classification **≤ 33** mg/kg bw/d	Liver, kidney, adrenals, nervous system NOAEL m: 30 ppm NOAEL f: 100 ppm	10 ppm: ↓ plasma cholinesterase activity (f: 24%) 30 ppm: ↓ plasma cholinesterase activity (f: 37%) 100 ppm: ↓ plasma cholinesterase activity (m: 44%, f: 58%),↓ erythrocyte cholinesterase activity (m: 20%, f: 13%)

6-month (diet) Dog, Beagle (6/sex/dose) In-house method No GLP (Anon. 1979, RAR Vol.3 B.6.3.3.2/01)	0, **200, 600** and 2000 ppm equal to: m: 0, 6.6, 24, 70 mg/kg bw/d f: 0, 6.0, 21, 63 mg/kg bw/d Guidance value for classification **≤ 50** mg/kg bw/d	Liver NOAEL m/f: 600 ppm	<u>200 ppm:</u> None <u>600 ppm:</u> - changes in haematological parameters (m: ↓ Hb at week 12/16: 11/8%) <u>2000 ppm*</u> ↓ body weight gain (m: 54% n.s., f: 46% n.s.) - changes in haematological parameters (m: ↓ Hb at week 12/16/24: 14/15/13%, ↓ erythrocyte count at week 8-24: 15-18%; f: ↓ Hb at week 20/24: 11/14%,↓ hematocrit at week 20: 10%, ↓ erythrocyte count at week 12-24: 12-19%) - changes in biochemical parameters (↑ alkaline phosphatase (m: 234%, f: 253%), ↓ plasma cholinesterase activity (f: 19%) - changes in organ weights (↑ liver (m: rel 79%, abs 56%; f: rel 65%, abs 43%)
1-year (diet) Dog, Beagle (6/sex/dose) EPA Guideline 83-1 GLP (Anon. 1988, RAR Vol.3 B.6.3.5.1/01)	0, **80, 400** and 2000 ppm equal to m: 0, 2.2, 11.4, 59 mg/kg bw/d f: 0, 2.6, 11.2, 62 mg/kg bw/d Guidance value for classification **≤ 25** mg/kg bw/d	Liver, pancreas, prostate NOAEL m/f: 400 ppm	<u>80 ppm:</u> None <u>400 ppm:</u> - histopathological changes (slight hepatocytic pigment (m, f))
4-week preliminary immunotoxicity study (diet) Mouse, CD-1, female (8/dose) US OPPTS 870.7800 No GLP (Anon. 2010, RAR Vol.3 B.6.8.1.2/01)	0, **100,** 2000 and 4500 ppm Equal to f: 0 19.6, 413, 749 mg/kg bw/d Guidance value for classification **≤ 300** mg/kg bw/d	Study used as supplementary data	<u>100 ppm</u> ↓ plasma cholinesterase activity (31%) No effects on the immune system
4-week immunotoxicity study (diet) Mouse, CD-1, female (10/dose) US OPPTS 870.7800 GLP (Anon. 2010, RAR Vol.3 B.6.8.1.2/02)	0, **500, 1500** and 4500 ppm Equal to f: 0, 91, 273, 811 mg/kg bw/d Guidance value for classification **≤ 300** mg/kg bw/d	Study used as supplementary data	<u>500 ppm</u> None <u>1500 ppm</u> None No effects on the immune system
90-day neurotoxicity	<u>Main phase</u>	NOAEL m/f: 1800	Main study:

study (diet) Rat, Alpk:APfS (Wistar-derived) (12/sex/dose (main study); 5/sex/dose/ time point (satellite group for acetyl cholinesterase measurements)) OECD TG 424 GLP (Anon. 2007, RAR Vol.3 B.6.7.1.2/01)	0, **300**, 1800 and 10000 ppm equal to m: 0, 20.6, 122.3, 735.7 mg/kg bw/d f: 0, 23.1, 135.8, 762.7 mg/kg bw/d <u>Satellite group</u> 0, **300**, 1800 and 10000 ppm equivalent to m: 0, 22.6, 130.2, 719.7 mg/kg bw/d f: 0, 24.3, 143.9, 817.5 mg/kg bw/d Guidance value for classification **≤ 100** mg/kg bw/d	ppm	<u>300 ppm:</u> None <u>1800 ppm*:</u> None Satellite study: <u>300 ppm:</u> ↓ erythrocyte cholinesterase (f: 8% at week 14) <u>1800 ppm*:</u> ↓ brain cholinesterase (f: 13% at week 14) ↓ erythrocyte cholinesterase (m: 10% at week 14, f: 14% at week 14)
2-year (diet) Rat, Fischer 344 CD®F (55/sex/dose (main study); 10/sex/dose (satellite group)) In-house method No GLP (Anon. 1985, RAR Vol.3 B.6.5.1/01)	0, **100, 300** and 1000 ppm equal to m: 0, 4.2, 12, 42 mg/kg bw/d f: 0, 4.8, 15, 49 mg/kg bw/d Guidance value for classification **≤ 12.5** mg/kg bw/d	None NOAEL m/f: ≥ 1000 ppm	<u>100 ppm</u> None <u>300 ppm:</u> None
104-week cholinesterase activity study (diet) Rat, Fischer 344 (30/sex/dose) In-house method No GLP (Anon. 1985, RAR Vol.3 B.6.5.1/02)	0, **100, 300** and 1000 ppm equal to m: 0, 4.1, 12, 42 mg/kg bw/d f: 0, 4.8, 15, 49 mg/kg bw/d Guidance value for classification **≤ 12.5** mg/kg bw/d	None Study considered as supplemental data	<u>100 ppm:</u> None <u>300 ppm:</u> None No effects on plasma, erythrocyte and brain cholinesterase activity
2-year (diet) Mouse, Crj:B6C3F1 (50/sex/dose (main study); 20/sex/dose (satellite group)) In-house method No GLP (Anon. 1983, RAR Vol.3 B.6.5.2/01)	0, **10, 50,** 250 and 1000 ppm equal to m: 0, 1.3, 6.4, 32.2, 134 mg/kg bw/d f: 0, 1.3, 6.9, 34.1, 137 mg/kg bw/d Guidance value for classification **≤ 12.5** mg/kg bw/d	Kidney, thymus, pituitary, nervous system NOAEL m/f: 50 ppm	<u>10 ppm:</u> None <u>50 ppm:</u> None

DERMAL			
21-day (6 h/d, 5 d/wk, 3 wks) Rabbit, New Zealand White/Hazleton Dutchland (5/sex/dose) EPA Guideline 158, 82-2 GLP (Anon. 1986, RAR Vol.3 B.6.3.6/01)	0, **30**, **300** and **1000** mg/kg bw/d vehicle: Acetone Guidance value for classification **≤ 1200** mg/kg bw/d	Skin, kidney NOAEL(systemic) m: ≥1000 mg/kg bw/d NOAEL(systemic) f: 300 mg/kg bw/d NOAEL (local) m/f: <30 mg/kg bw/d	<u>30 mg/kg bw/d:</u> - dermal irritation (erythema (m, f)) - histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration (m, f)) <u>300 mg/kg bw/day:</u> - dermal irritation (erythema (m, f)) ↓plasma cholinesterase activity (f: 29%) - histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration (m, f)) <u>1000 mg/kg bw/day:</u> - dermal irritation (erythema (m, f)) - changes in haematological parameters (↑eosinophil value (m: 100%)) ↓plasma cholinesterase activity (f: 25%) - changes in organ weights (↑ kidney (f) rel 20%) - histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration (m, f))

* dose level is above the guidance value for classification, but presented here as it is relatively close to the guidance value

Liver and kidney were identified as the main target organs in the studies with repeated dosing. Effects observed at dose levels relevant for STOT RE included changes in liver and kidney weight (mostly without histopathological findings), as well as changes in some biochemical and haematological parameters. The DS considered none of these effects of sufficient severity for classification for STOT RE.

In addition to the above, an inhibitory effect on acetylcholinesterase activity was observed in most of the repeated dose studies. In rats and mice, the activity of plasma, erythrocyte and brain acetylcholinesterase was affected while in dogs and rabbits only plasma acetylcholinesterase activity was affected. The effects on acetylcholinesterase activity were not accompanied by clinical signs indicative of neurotoxicity in any of the species, nor with neuropathology findings in the rat 90-day neurotoxicity study. In deciding on the classification, the DS considered that the reduction in acetylcholinesterase activity should be ≥ 20% in order to be considered adverse and to possibly warrant classification for STOT RE when observed at dose levels below the guidance values for classification. However, when 1) a 20% or greater reduction was seen in plasma acetylcholinesterase only and erythrocyte and brain acetylcholinesterase were unaffected, or 2) the reduction in erythrocyte and/or brain acetylcholinesterase activity was ≥ 20% but without clinical signs present, the DS considered the effect not severe enough to warrant classification. In most studies the magnitude of acetylcholinesterase activity inhibition was less than 20% at doses within the critical range of doses for classification, therefore not warranting classification. The only

exceptions were the oral 9-month mouse study and the dermal 21-day rabbit study, but the DS considered that for these studies criterion 2 and 1 (as mentioned above) apply, respectively.

Overall, the DS proposed no classification for STOT RE.

Comments received during public consultation

One MSCA noted that during the Pesticides Peer Review 162 the majority of experts agreed that a reduction in cholinesterase activity of approximately 20% might not be relevant in the absence of neurotoxic effects. However, as STOT RE can, in principle, be based on "serious changes in biochemistry", the MSCA suggested that this issue be addressed. The DS responded that classification with STOT RE (H373) may be relevant, given the >20% reduction in acetylcholinesterase activity at dose levels within the critical range for Cat. 2 classification in the 9-month mouse study.

Assessment and comparison with the classification criteria

In the available repeated dose studies, treated animals showed a variety of effects. At dose levels relevant for STOT RE classification, these included effects on liver in rats and dogs (organ weight, changes in some biochemical parameters and, in dogs only, slight hepatocytic pigment), effects on kidney in rats and rabbits (organ weight), effects on some haematological parameters in dogs, and effects on acetylcholinesterase activity in rats, mice, dogs and rabbits. With respect to the effects on liver in rats and dogs, there was, however, no clear evidence of organ dysfunction. With respect to the effects on kidney in rats and rabbits, it is noted that the organ weight changes were not accompanied by histopathological changes. Finally, the changes in some of the haematological parameters in dogs were minor. RAC agrees with the DS that these effects are not sufficiently severe to fulfil the classification criteria.

Concerning the effects on acetylcholinesterase activity as observed in various studies, RAC notes that according to the recommendations of the WHO JMPR guidance, clinical signs and inhibition (≥ 20%, statistically significant and fitting a dose- or time-related trend) of brain cholinesterase activity are considered to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterase activity. Inhibition (≥ 20%, statistically significant and fitting a dose- or time-related trend) of erythrocyte acetylcholinesterase is also considered to be an adverse effect, which can be used as a surrogate for brain acetylcholinesterase inhibition when data on this enzyme are not available. Inhibition of plasma acetylcholinesterase is only considered as an indication of adversity. Taking this into account, as well as recognising the adversity of brain acetylcholinesterase inhibition in particular and that the degree of acetylcholinesterase inhibition that can be tolerated without clinical symptoms can vary between individuals and substances, RAC considers a statistically significant and dose- or time-related inhibition of acetylcholinesterase of ≥ 20% in brain (or in erythrocytes, as a surrogate when no data on the brain are available) to meet the criteria for classification (in particular CLP Annex I 3.9.2.7.3(c)), even when it is not accompanied by clinical signs.

Clinical signs typical for cholinergic effects were not observed in any of the repeated dose studies. Neither were statistically significant reductions of 20% or more of brain or

erythrocyte acetylcholinesterase activity observed in these studies at dose levels relevant for classification, except for one study. In this particular study, the 9-month mouse study, a 20% reduction of erythrocyte acetylcholinesterase activity was noticed in male animals upon treatment with 100 ppm (corresponding to 12.2 mg/kg bw/d). This increased to a 55% reduction at the next higher (top) dose of 3000 ppm (corresponding to 513 mg/kg bw/d), but this dose is well above the (extrapolated) guidance value of 33 mg/kg bw/d. In the mouse study, brain acetylcholinesterase activity was also measured: at 3000 ppm, it was inhibited in the males (by 25%), but there was no dose-related trend since at the dose level relevant for classification (100 ppm) the activity was in fact increased by 19%. Overall, RAC notes that the percentage inhibition of erythrocyte acetylcholinesterase activity in this 9-month mouse study was at the cut-off of 20% at a dose level just below the (extrapolated) guidance value for classification, that brain acetylcholinesterase activity was not inhibited at this dose level but increased instead, and that no adverse effects on brain or erythrocyte acetylcholinesterase activity (nor clinical signs) were observed in any of the other repeated dose studies (including a 2-yr mouse study) at dose levels relevant for classification. RAC therefore considers the sole finding of a 20% reduction in erythrocyte acetylcholinesterase activity in one study in one sex only, without inhibitory effect on brain acetylcholinesterase activity, insufficient evidence for classification.

Overall, RAC agrees with the DS that **classification for STOT RE is not warranted** for tolclofos-methyl.

10.13 Aspiration hazard

Table 56: Summary table of evidence for aspiration hazard

No data

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

Not relevant as no data is available

10.13.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for aspiration hazard

10.13.3 Conclusion on classification and labelling for aspiration hazard

No classification is proposed for tolclofos-methyl

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

All the information on ready biodegradability are taken from the RAR and list of endpoints for toclofos-metyl, October 2017.

A significant loss of test substance due to volatilisation was observed in water/sediment study and in particular in the study on mineralisation in water. This led to difficulties to correctly estimate the degradation rate. Tolclofos-methyl has a vapour pressure of 8.77 x 10^{-4} Pa at 20ºC. Based only on this value a pronounced volatilisation may not be expected. However, tolclofos-methyl has a relatively low water solubility and therefore the value of Henry's law constant (calculated as vapour pressure x mol. weight x water sol.$^{-1}$) is relatively high at 0.37 Pa m^3 mol^{-1} at 20°C. The substance therefore has a tendency to volatilise from water and moist surfaces.

Table 57: Summary of relevant information on ready degradability

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>OECD 301 C</td><td>Ready biodegradability:
After 7 days -2 percantage degradation and after 14 days -1 percantage degradation (BOD-B/TODx100%) was found in the active sludge</td><td>Tolclofos-methyl is not readliy degradable

Reliability 1</td><td>Nambu, K., et al (1984)</td></tr>
<tr><td>EEC Method C.7</td><td>Hydrolytic degradation of the active substance and metabolites>10%:
pH 4.0: DT_{50} 126 d at 20°C
(1st order, extrapolated from data at 50, 62, 74°C)

DM-TM: max 48% AR (after 5 d at 50°C)
ph-CH_3: not detected

pH 7.0: DT_{50} 97 d at 20°C
(1st order, extrapolated from data at 50, 62, 74°C)

DM-TM: max 65% AR (after 5 d at 50°C)
ph-CH_3: not detected

pH 9.0: DT_{50} 102 d at 20°C
(1st order, extrapolated from data at 50, 62, 74°C)

DM-TM: max 46% AR (after 5 d at 50°C)
ph-CH_3: max 12 % AR (after 5 d at 50°C)</td><td>Tolclofos-methyl is not proned to hydrolysis in the environment.

Reliability 1</td><td>Lewis C.J, 2001b</td></tr>
<tr><td>OECD TG No 309</td><td>Aerobic mineralisation in surface water:
Tolclofos-methyl
<table>
<tr><th rowspan="2">System identifier (indicate fresh, estuarine or marine)</th><th rowspan="2">pH water phase</th><th rowspan="2">pH sed [a)]</th><th rowspan="2">t. °C [b)]</th><th colspan="2">DT_{50} /DT_{90} whole sys. (suspended sediment test)</th><th colspan="2">DT_{50} /DT_{90} Water (pelagic test)</th></tr>
<tr><th>At study temp</th><th>Normalised to x °C[c)]</th><th>At study temp</th><th>Normalised to x °C[c)]</th></tr>
<tr><td>European pond water – “open system”</td><td>8.12</td><td>-</td><td>21 ± 0.3</td><td>-</td><td>-</td><td>Not calculated</td><td>-</td></tr>
<tr><td>European pond water – closed system</td><td>8.03</td><td>-</td><td>21 ± 0.3</td><td>-</td><td>-</td><td>Not calculated</td><td>-</td></tr>
</table>
a) Measured in water
b) Temperature of incubation
c) Normalised using a Q10 of 2.58 to the temperature of the environmental media at the point of sampling. (note temp of x should be stated).</td><td>The volatile losses of the tolclofos-methyl during the aerobic mineralization study make interpretation of the results difficult and it was not possible to determine degradation rates.

Reliability 3</td><td>Adam (2015)</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td></td><td>

Metabolite DM-TM

<table>
<tr><th rowspan="2">System identifier (indicate fresh, estuarine or marine)</th><th rowspan="2">pH water phase</th><th rowspan="2">pH sed [a)]</th><th rowspan="2">t. °C [b)]</th><th colspan="2">DT_{50} /DT_{90} whole sys. (suspended sediment test)</th><th colspan="2">DT_{50} /DT_{90} Water (pelagic test)</th></tr>
<tr><th>At study temp</th><th>Normalised to x °C [c)]</th><th>At study temp</th><th>Normalised to x °C [c)]</th></tr>
<tr><td>European pond water – “open system”</td><td>8.12</td><td>-</td><td>21 ± 0.3</td><td>-</td><td>-</td><td>Not calculated</td><td>-</td></tr>
<tr><td>European pond water – closed system</td><td>8.03</td><td>-</td><td>21 ± 0.3</td><td>-</td><td>-</td><td>Not calculated</td><td>-</td></tr>
</table>

a) Measured in water

b) Temperature of incubation

c) Normalised using a Q10 of 2.58 to the temperature of the environmental media at the point of sampling. (note temp of x should be stated).

<table>
<tr><th colspan="6">Mineralisation and non extractable residues (for parent dosed experiments)</th></tr>
<tr><th>System identifier (indicate fresh, estuarine or marine)</th><th>pH water phase</th><th>pH sed</th><th>Mineralisation x % after n d. (end of the study).</th><th>Non-extractable residues. max x % after n d (suspended sediment test)</th><th>Non-extractable residues. max x % after n d (end of the study) (suspended sediment test)</th></tr>
<tr><td>European pond water – “open system”</td><td>8.12</td><td>-</td><td>≤ 0.5% after 61 days</td><td>-</td><td>-</td></tr>
</table>

</td><td></td></tr>
<tr><td>SETAC, 1995
FOCUS Kinetics (2006; 2014)</td><td>

Degradation in water/sed system:

<table>
<tr><th rowspan="2">System</th><th rowspan="2">pH water phase</th><th rowspan="2">pH sed</th><th rowspan="2">t. °C</th><th colspan="4">Total system (A4)</th></tr>
<tr><th>Kinetic model</th><th>χ^2, error-%</th><th>**$DegT_{50}$**</th><th>**$DegT_{90}$**</th></tr>
<tr><td>Emperor Lake</td><td>6.6</td><td>6.1</td><td>20± 2</td><td>SFO</td><td>26.7</td><td>64.1</td><td>213</td></tr>
</table>

</td><td>Tolclofos-methyl is not readily biodegradable in two tested systems

Reliability 1</td><td>Lewis,C.J (2001c)</td></tr>
</table>

Method	Results								Remarks	Reference
	Mill Stream Pond	7.9	7.2	20±2	SFO	12.3	32.2	107		

11.1.1 Ready biodegradability

Reference:	Nambu, K., Itoh, K., Yamada, H., Miyamoto, J. (1984) Biodegradation test: Tolclofos-methyl (Rizolex)
Report No.:	QM-40-0013
Guideline:	OECD TG No 301 C
GLP:	No
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No.:	not stated
Purity:	>99%
Reference substance:	Aniline
Test concentration:	100 ppm (w/v)
Test system:	BOD meter, basal culture medium that contained 30 ppm (w/v) of activated sludge on a dry weight basis. The test solution was stirred vigorously and BOD was measured continuously.
Temperature:	25±1°C
Test period:	14 days

Results

The percentage degradation of tolclofos-methyl and aniline were calculated from the BOD, as shown in Table 8.2.2.1-1. Since the percentage degradation of aniline exceeded 40% after 7 days and 65% after 14 days, the test was regarded as valid. From the percentage degradation of tolclofos-methyl, it is clear that tolclofos-methyl is not biodegradable under the conditions tested.

Table 58: Biodegradation test results

Incubation time (Days)			7		14	
	Application amount (mg)	TOD (mg)	Oxygen consumption (mg)	Percentage degradation (%)*	Oxygen consumption (mg)	Percentage degradation (%)
TM+sludge	30	40	2.5	-2	4.2	-1
Aniline+sludge	30	90	66	69	78	81
Sludge only	-	-	3.1	-	4.5	-

*: Percentage degradation = (BOD – B)/TOD x 100 (%)

BOD: Biochemical oxygen demand (experimental) (mg) of the test compound measured on the BOD curve

B: Oxygen consumption (experimental) (mg) of basal culture medium to which the inoculum is added measured on the BOD curve

TOD: Theoretical oxygen demand (theoretical) (mg) required when the test compound is completely oxidised

Conclusion

The study was well performed according to OECD 301 C guidelinesa and well reported according to RMS comments. The study indicates that tolclofos-methyl is not readily biodegradable.

11.1.2 Hydrolysis

Reference:	Lewis C.J. (2001b) (^{14}C)-Tolclofos-methyl: Hydrolytic stability
Report No.:	QM-0051
Guideline:	EEC Method C.7
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	^{14}C-Tolclofos-methyl (^{14}C-phenyl-labelled)
Lot/Batch No:	CP-2427
Radiochemical purity:	98.9%
Test system/test conditions:	The test substance was applied in acetonitrile (23.5 µl) to degassed, nitrogen bubbled and sterilised buffer solutions (3 mL) in glass vials. The samples were incubated at the relevant temperature for up to 5 days in darkness. Test concentration was 0.2 mg/l.
Temperature:	50±0.5°C, 62±0.5°C and 74±0.5°C
pH (buffer systems):	4.0 (0.05 M potassium hydrogen phthalate), 7.0 (0.05 M TRIS-maleic acid) and 9.0 (0.05 M Sörensen buffer)
Sampling and	Duplicate samples were removed for analyses at 4-7 sampling points, depending on test temperature. Radioactivity was measured by LSC and an

analyses:	aliquot was mixed with acetonitrile and analysed by HPLC. Where necessary, the identity of degradation products was confirmed by TLC. At the last time point in the 50°C incubation (all pHs) and at one time-point in the 62°C incubation (10.6 hours at pH 9), rinses of test vessels were combined, concentrated under nitrogen, the radioactivity re-determined and the extracts analysed by HPLC. Reference standards to identify degradation products: TM-CH_2OH, TM-COOH, TMO, TMO-CH_2OH, TMO-COOH, DM-TM (potassium salt), DM-TM-CH_2OH (potassium salt), DM-TM-COOK (potassium salt), DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOK (potassium salt), ph-CH_3 (DCMP), ph-CH_2OH, ph-COOH, DC-ph-CH_3, Me-ph-CH_3, *p*-Cresol, and DM-TM-SCH_3.
Kinetic evaluation:	Half-lives were calculated using SFO equation (non-linear regression). Half-lives at 20 and 25°C were calculated using the Arrhenius equation and experimental data from 50, 62 and 74°C.

Results

Recovery of applied radioactivity was >90% in all samples. As shown in the table below the single major hydrolysis product at pH 4 and 7 was DM-TM, while also ph-CH_3 was identified at pH 9. After 5 days at 50°C DM-TM accounted for 48%, 65% and 46% of applied radioactivity at pH 4, 7 and 9 respectively. ph-CH_3 accounted for 11.5% of applied radioactivity after 5 days at pH 9 and 50°C. Up to 5% AR was recovered from the unit rinses. At all pH values analysis of this radioactivity revealed that the majority of the radioactivity was tolclofos-methyl, with DM-TM and ph-CH_3 present in very small quantities.

Table 57: Tolclofos-methyl: Hydrolysis half-lives at pH 4, 7 and 9 and different temperatures (single first order kinetics, non-linear regression, all r^2 ≥0.988)

Temperature, °C	pH	DT_{50}, hours	DT_{50}, days
50	4	97	4.0
	7	61	2.5
	9	76	3.2
62	4	32	1.3
	7	17	0.7
	9	24	1.0
74	4	9.6	0.4
	7	5.1	0.2
	9	7.3	0.3
20 [a]	4	3035	126
	7	2336	97
	9	2446	102
25 [a]	4	1638	68
	7	1208	50
	9	1310	55

[a] Calculated using the Arrhenius equation and data from the 50, 62 and 74°C experimental data.

Conclusion

The RMS commented that the study was accepted in the DAR (2003) and is considered acceptable also for the purpose of renewal. Based on the study, EFSA concluded (2005) that tolclofos-methyl is not prone to hydrolysis under environmental conditions.

Overall, it can be concluded that hydrolysis will not be a significant degradation pathway of tolclofos-methyl.

11.1.3 Other convincing scientific evidence

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

Aerobic mineralization in surface water

A new study on aerobic mineralisation was submitted for the purpose of renewal (Adam, 2015).

Reference:	Adam (2015) [^{14}C]-tolclofos-methyl - aerobic mineralisation in surface water - simulation biodegradation test
Report No.:	QM-0079
Guideline:	OECD TG No 309
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Material and methods:	
Test material:	[Phenyl-U-^{14}C] Tolclofos-methyl
Lot/Batch No:	CFQ42051
Radiochemical purity:	98.5 %
Test concentration:	Target concentrations as follows: Low dose: 10 µg/l High dose: 100 µg/l High dose (sterile): 100 µg/l The stock solution was prepared in methanol.
Test system:	Due to the volatility of the test item, two separate test systems were performed for this study. The first system consisted of an open gas-flow-system, referred to in the study as the "open test system". After treatment, samples were connected to a trapping system equipped with two absorption traps (ethylene glycol and 2N NaOH) to trap organic volatiles and $^{14}CO_2$, respectively. Flask were aerated with moistened air. Tubing was equipped with polyurethane foam traps. The second system consisted of closed test vessels with minimum headspace. The closed systems did not have any traps. Benzoic acid was used as a reference substance to check the sufficiency of microbial activity of the chosen surface water. In order to examine possible adverse effects of methanol additional

	control samples were prepared within the benzoic acid system. One set of replicates was prepared without the addition of methanol and a second set was prepared with the same amount of methanol as in the samples containing the test item. These experiments were conducted each in duplicate with freshly sampled surface water for both the open and closed test systems.
Test conditions:	Samples were incubated at 21 ± 0.3°C under continuous stirring in the dark.

Results

A European pond system was studies using two closed systems (with and without volatile traps). Evolution of CO_2 was minimal and organic volatiles were detected up to 61.3 % AR (identified as Tolclofos-methyl). These losses occurred regardless of concentration, presence of microbial activity, test system and analysis techniques. Up to four metabolites were detected. The major metabolite DM-TM was observed with a maximum of 13.5 % of AR on day 61. The other metabolites (TMO and two unknowns) were considered as minor metabolites (< 5 %). However, it is not possible to gauge what effect there might have been on the maximum observed levels had tolclofos-methyl remained in the aqueous phase. It was concluded that this study type is not suitable for a volatile compound like tolclofos-methyl and the RMS does not believe repeating the study would yield a different outcome. The RMS therefore accepts the study as such but considers some of the results as highly uncertain.

Conclusion

The performance of this study was hampered by the volatile nature of the test substance. Evolution of CO_2 was minimal and the losses during the study make interpretation of the results difficult. These losses occurred regardless of concentration, presence of microbial activity, test system and analysis techniques. The author concluded that this study type is not suitable for a volatile compound like tolclofos-methyl which is also indicated in the OECD Guideline 309 regarding volatile compounds. The RMS is inclined to agree and does not believe repeating the study would yield a different outcome. The RMS therefore accepts the study as such but considers some of the results as highly uncertain.

With regard to the degradation pattern, it is difficult to draw conclusions based on the data provided. The metabolite DM-TM reached maximum amounts of 13.5 % AR while the other metabolites, TMO, M5 and M6, did not exceed 5 % AR throughout the study. However it is not possible to gauge what effect there might have been on the maximum observed levels had tolclofos-methyl remained in the aqueous phase.

The conclusion is that the volatile losses of the tolclofos-methyl during the aerobic mineralization study make interpretation of the results difficult and it was not possible to determine degradation rates.

Water/sediment study

One study investigating the degradation in water/sediment systems (Lewis, 2001c) was available. The study was first evaluated in the DAR (2003) and it was considered acceptable also for the renewal of the approval.

Reference:	1. Lewis, C.J. (2001c) (^{14}C)-Tolclofos-methyl: degradation and retention in water-sediment systems
	2. Weimann, T., Lobe.I. (2015c) assessment of degradation kinetics of tolclofos-methyl and its metabolites DM-TM and ph-CH_3 in water/sediment systems under laboratory conditions according to the recommendations of the focus report on degradation kinetics (2006, 2014).

Report No.:	1. QM-0050
	2. QM-0087
Guideline:	1. SETAC, 1995
	2. FOCUS kinetics (2006; 2014)
GLP:	1. Yes
	2. Not subject to GLP
Previous evaluation:	1. In DAR (2003)
	2. Submitted for the purpose of renewal
Material and methods:	
Test material:	[Phenyl-^{14}C]tolclofos-methyl
Lot/Batch No:	CP-2427
Radiochemical purity:	98.9%
Test concentration:	64 µg per unit (0.67 µg/mL), equivalent to 2 kg a.s./ha assuming uniform distribution in a water body to a depth of 30 cm.
Test system and conditions:	Route and rate of degradation was studied in two water/sediment systems, Mill Stream pond and Emperor Lake (see Table 8.2.2.3-1 for characteristics). The test was performed in glass cylinders of 4.5 cm diameter in darkness with a sediment layer of 2.5 cm and a water layer of 6 cm. Moistened CO_2 free air was drawn over the water surface and the systems were equilibrated for 56 days in the dark at 20±2°C before application of test substance. Air drawn over the surface was passed through traps to capture any evolved volatiles (polyurethane foam bung, ethanediol, 2% paraffin in xylene and 2 M sodium hydroxide solution). Oxygen, pH and redox potential measurements were made during acclimatisation and at each time point during the study. Total N and total P were measured initially and at the end of the study. Microbial biomass of the sediment was determined at the start and after incubation.

Results

Two European water/sediment systems (Millstream Pond and Emperor Lake) were examined for 100 days. Partitioning from the water to the sediment was relatively rapid with 6.4 and 12% AR of the parent remaining by day 3 in the water phase in the Mill stream pond system and the Emperor Lake system, respectively. The levels of radioactivity peaked in the sediments on day 7 of the incubation for both systems. Un-Extractable residues in sediment increased to a maximum of 26 % and 35 % AR at 62 and 76 days after application. Carbon dioxide increased throughout the study to a maximum of 36 to 53% AR by the end of the study. A significant amount of tolclofos-methyl was detected as volatiles with over 30% of applied radioactivity detected at certain time points. The variation in the amount trapped appeared to be due to the variation in the rate of flow of air through the systems. One major metabolite (DM-TM) and several minor metabolites (DM-TMO, ph-CH_3 and TM-COOH) were detected in both water and sediment systems. The metabolite DM-TM was detected in significant quantities in both water and sediment, occurring at respective maximums of 11 and 13% AR after 30 days of incubation.

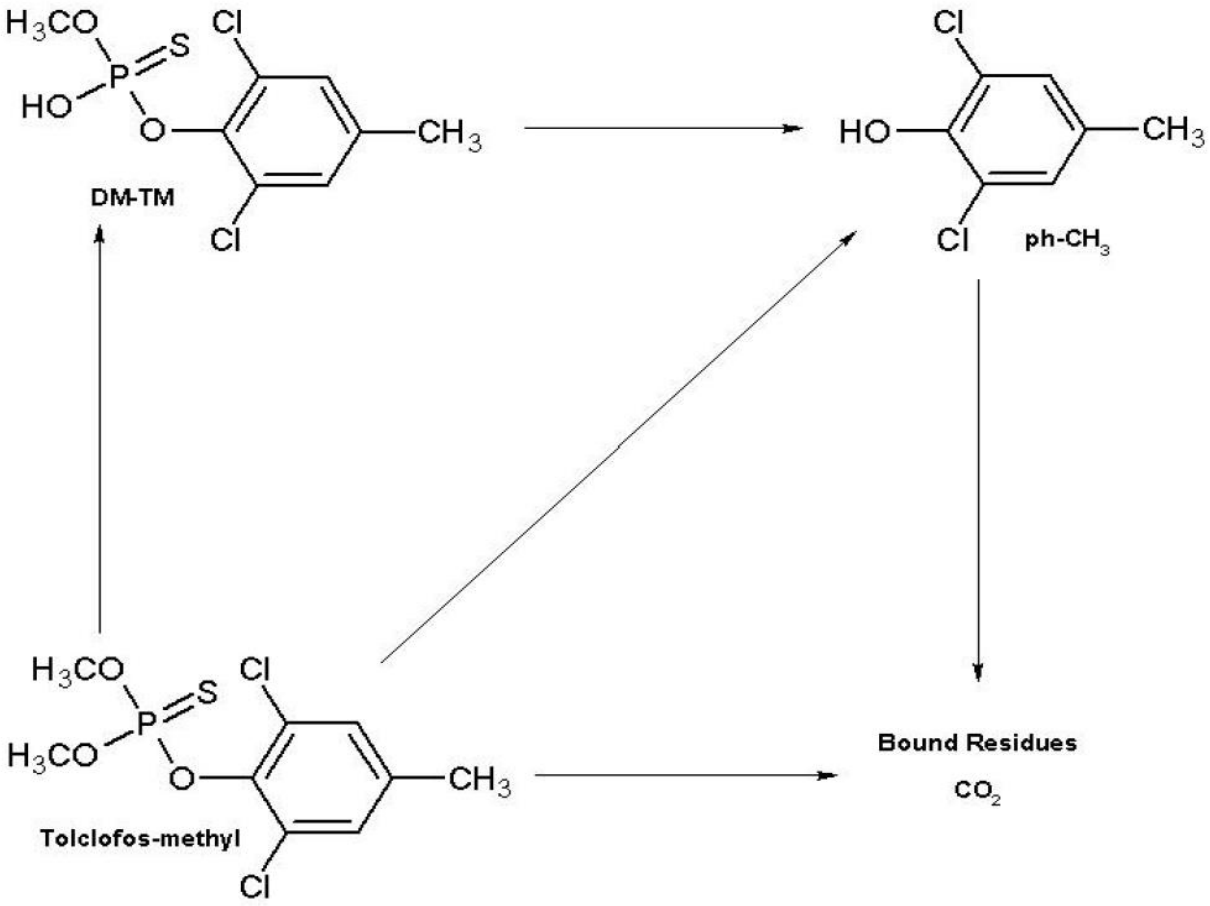


Figure Proposed pathway for degradation in water

Table 59: Water / sediment study (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.2.3 and Regulation (EU) N° 284/2013, Annex Part A, point 9.2.2)

Tolclofos-methyl	Distribution (e.g. max in water 57.9 after 0 d. Max. sed 72.9 % after 3 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Emperor Lake	6.6	6.1	20±2	64.1/213	26.7	Not reliable	20.1	38.5/127.8	12.6	SFO
Mill Stream Pond	7.9	7.2	20±2	32.2/107	12.3	0.37/2.7	8.7	20.9/69.3	3.8	SFO
Geometric mean at 20°C[b)]				45.4						

[a)] Measured in water

[b)] Normalised using a Q10 of 2.58

Metabolite DM-TM	Distribution: Max in total system 23.4 % after 30 days. Max in water 10.7% after 30 d. Max. sed 12.7 % after 30 d). Formation fraction: - Precursor: from parent.									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Emperor Lake	6.6	6.1	20±2	Not reliable	27.8	-		-		SFO
Mill Stream Pond	7.9	7.2	20±2	Not reliable	0.24	-		-		SFO
Geometric mean at 20°C[b)]										

[a)] Measured in water

[b)] Normalised using a Q10 of 2.58

* Only 3 datapoints available in decline phase yielding DT_{50} /DT_{90} = 10.6/35.2 days.

Metabolite ph-CH_3	Distribution: Max in total system 6.5 % after 30 days. Max in water 0.5 % after 30 d. Max. sed 6 % after 30 d. Formation fraction: - Precursor: from parent and DM-TM.									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Emperor Lake	6.6	6.1	20±2	Not reliable	17.1	-		-		SFO
Mill Stream Pond	7.9	7.2	20±2	Not reliable	8.9	-		-		SFO
Geometric mean at 20°C[b)]										

[a)] Measured in water

[b)] Normalised using a Q10 of 2.58

* Only 4 datapoints available in decline phase for Emperor Lake yielding DT_{50} /DT_{90} = 30.3/100.7 days. Only 3 datapoints available in decline phase for Mill Stream pond yielding DT_{50} /DT_{90} = 35.0/116.4 days.

Mineralisation and non extractable residues (from parent dosed experiments)					
Water / sediment system	pH water phase	pH sed	Mineralisation x % after n d. (end of the study).	Non-extractable residues in sed. max x % after n d	Non-extractable residues in sed. max x % after n d (end of the study)
Emperor Lake	6.6	6.1	36% after 100 d	35% after 72 d	20% after 100 d
Mill Stream Pond	7.9	7.2	53% after 100 d	26% after 62 d	20% after 100 d

Conclusion

Tolclofos-methyl is not rapidliy biodegradable in the two different water/sediment test systems and the results on the metabolites where not reliable enough for classification purpose.

11.1.3.2 Photochemical degradation

For the first approval of tolclofos-methyl two studies addressing direct aquatic photolysis were available (Takahashi & Katagi, 1988; Takahashi, 1981). The study by Takahashi (1981) on quantum yield is no longer considered acceptable. One new study (Curtis-Jackson, 2014) on direct photolysis was submitted to support the renewal of the approval. This new study only investigated the rate of photochemical transformation of tolclofos-methyl. Takahashi & Katagi (1988) also analysed for photolysis products.

Reference:	Takahashi, N., Katagi, T. (1988) Photolysis of tolclofos-methyl in water
Report No.:	QM-80-0024
Guideline:	US EPA guideline §161-2
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	^{14}C-Tolclofos-methyl (^{14}C-phenyl-labelled)
Lot/Batch No.:	C-B5-127
Radiochemical purity:	99.0 %
Test system/test conditions:	Direct photochemical transformation of tolclofos-methyl was studied in pure water at pH 7 over 30 days. A xenon arc lamp was used, with wavelengths < 290 nm filtered off. The study was run with 24 h light per day, which was equivalent to 12 h exposure to natural sunlight at 40°N latitude. One single test vessel was exposed and one control vessel was maintained in darkness. Test concentration was 0.2 mg/l. Ethyl acetate was used as solvent, and the solvent was removed by purging with nitrogen gas before addition of buffer.
Temperature:	25 ± 1°C
pH (buffer systems):	HEPES [3-(4-(2-hydroxyethyl)-1-piperadinyl)-1-propane sulfonic acid] buffer (pH 7.0). The aqueous buffer was sterilised by autoclaving before preparation of the test solution.
Sampling and analyses:	Aliquots from the test vessels were removed immediately after dosing, and at 1, 3, 5, 7, 14, 21 and 30 days. Before sampling, carbon dioxide-free air was passed over the water surface to trap any volatile ^{14}C in a polyurethane foam plug and NaOH aqueous solution. The removed samples were acidified to pH 2 and extracted with ethyl acetate. The radioactivity in organosoluble and aqueous fractions or gel regions scraped from TLC plates was determined by LSC. Breakdown products were separated by TLC and tentatively identified by co-chromatography with non-labelled reference compounds. The identification of photoproducts occurring at >10 % AR was confirmed by HPLC. Reference standards to identify degradation products: TMO, ph-CH_3, DM-TM, DM-TMO and TM-SCH_3.
Kinetic evaluation:	Half-lives were estimated with single first order equation.

Results

The results for the exposed sample and the dark control are shown in the table below. $^{14}CO_2$ and other volatiles are not shown since amounts were negligible (≤ 0.1% AR). The degradation of tolclofos-methyl appeared to be slightly enhanced by irradiation. First order half-lives were estimated to 38 days under irradiated conditions (r^2 0.94) and 77 days under dark conditions (r^2 0.74).

Table 60: Tolclofos-methyl: Distribution and characterisation of irradiated samples and dark control over 30 days.

	Sampling time, days							
	0	**1**	**3**	**5**	**7**	**14**	**21**	**30**
IRRADIATED SAMPLE								
Extracted ^{14}C	101	104	106	99	101	95	90	87
Tolclofos-methyl	100	101	104	97	99	78	66	60
TMO	-	0.2	0.2	0.1	0.1	0.6	0.7	0.8
ph-CH_3	-	-	-	-	-	-	-	1.3
DM-TM	-	0.7	1.6	0.4	0.4	9.7	12	13
DM-TMO	-	-	-	-	-	-	-	0.9
TM-SCH_3	-	-	-	-	-	0.1	-	0.2
Others	1.8	2.1	3.4	1.6	1.6	7.4	8.8	11
Unextracted ^{14}C	< 0.1	0.2	0.3	0.4	0.6	2.4	3.6	7.4
Total ^{14}C	101	105	107	99	101	97	93	95
DARK CONTROL								
Extracted ^{14}C	101	105	103	100	95	98	91	91
Tolclofos-methyl	100	102	98	98	94	90	74	80
TMO	-	0.3	0.3	0.2	0.2	0.7	1.4	0.7
ph-CH_3	-	-	-	-	-	-	-	1.5
DM-TM	-	0.7	1.7	0.2	0.2	3.8	7.3	5.4
DM-TMO	-	-	-	-	-	-	-	-
TM-SCH_3	-	-	-	-	-	-	-	-
Others	1.8	1.9	3.1	1.0	1.0	2.5	8.2	3.7
Unextracted ^{14}C	< 0.1	0.1	0.2	0.2	0.6	1.5	2.4	4.6
Total ^{14}C	101	106	103	100	96	99	93	96

The RMS comment was that the study was accepted in the DAR (2003). The DAR noted that no correction was made for the degradation that occurred in the dark control in the calculation of half-life. It can be added that half-lives were extrapolated beyond the study duration and therefore uncertain. Lack of replicates also adds to the uncertainty of the results. More reliable data on rate of aquatic photolysis has been submitted to

support the renewal of the approval (Curtis-Jackson, 2014). However, the results from Takahashi & Katagi (1988) are considered sufficient to determine the route of degradation. Withdrawing aliquots of test solutions at each sampling time (as in this study) is acceptable however sacrificial sampling of entire photolysis cells at each sampling time is generally preferred (OECD TG No. 316, 2008).

Reference:	Curtis-Jackson, P. (2014) Tolclofos-methyl: Aqueous Photolysis and Determination of the Quantum Yield of Tolclofos-methyl
Report No.:	QM-0074
Guideline:	OECD TG No 316 (2008); US EPA 540/9-82-021, section 161-2 (1982); US EPA OCSPP 835.2210 (1998) ; US EPA OCSPP 835.2240 (2008) Calculation of quantum yield done in accordance with ECETOC Technical Report No. 12 (1984)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	^{14}C-Tolclofos-methyl (^{14}C-phenyl-labelled)
Lot/Batch No.:	CFQ41873 (unlabelled test item: C110225G)
Radiochemical purity:	99.7%
Test concentration:	0.36 mg/l (nominal)
Test system/test conditions:	The rate of direct phototransformation of tolclofos-methyl was investigated by two different approaches: 1) from experimental data from irradiated samples and dark controls, and 2) by calculation from determined quantum yield. There were no attempts to identify or quantify any transformation products. Tolclofos-methyl in solvent was dissolved into sterilized water in sterilized borosilicate photolysis tubes with quartz lids. Preliminary studies had indicated that after 48 hours up to 10% AR was bound to the walls of the photolysis test vessels which was not easily removed using organic solvents. The final study was therefore run with two different concentrations of solvent; acetonitrile at proportions of 10% v/v and 1% v/v. It was proposed that higher than recommended percentage of organic solvent would allow the test substance to maintain in solution without photosensitizing the test system since the acetonitrile:water mixtures would not significantly absorb photons in the 300 nm region.

The samples were continuously irradiated with xenon arc lamp and wavelengths
< 290 nm removed. The spectral energy distribution (λ; 290-800 nm) of the light source was recorded. Temperature was 25±1°C.

Dark control samples were prepared and incubated under identical conditions but in the absence of light.

Sampling and analysis: Duplicate vessels from the irradiated and the dark control environments were sacrificed on days 0, 3/4, 7, 10/11, 14 and 17/18. Aliquots were removed and analysed for tolclofos-methyl by LC/MS.

Sterility was assessed at the start and end of the exposure period in the irradiated and the dark control test solutions for both test conditions. Aliquots were distributed onto agar plates which were incubated at room temperature for 5 days (10% solvent samples) or 35 days (1% solvent samples).

pH was measured for each sacrificed test vessel.

Kinetic evaluations: Half-lives were determined using first order equation (linear regression). The final rate constant was corrected for degradation observed in dark controls.

Additionally the quantum yield (i.e. the fraction of absorbed light that results in a photoreaction at a given wavelength, Φ, dimensionless) of tolclofos-methyl was calculated. The result was used to calculate photolytic rate constants for 20-50°N latitude and for spring, summer, autumn and winter, according to the equation:

$$k_{pE} = \Phi_E \cdot \sum_{800}^{290} \varepsilon_\lambda \cdot L_\lambda$$

Where:
k_{pE} = first-order rate constant for photolysis in natural water (d^{-1}),
Φ_E = quantum yield of tolclofos-methyl independent of wavelength,
ε_λ = molar absorptivity ($molar^{-1}\ cm^{-1}$), and
L_λ = solar irradiance in water (10^{-3} einsteins $cm^{-2}\ day^{-1}$).
Solar irradiance (L_λ) in water at different seasons and wavelengths were based on GC SOLAR Program (US EPA guideline from 1998 see above).
Molar absorptivity (ε_λ) was determined by spectrophotometry for the

wavelengths 297.5 to 800 nm.

For the calculation of quantum yield the number of incident photons (P_{inc}) was obtained from data generated from actinometric measurements. The actinometer solution was illuminated under the same condition as those used for tolclofos-methyl. Actinometric photo-reactions occur with a known quantum yield (independent of the wavelength), which allowed P_{inc} to be determined. The actinometer was prepared with p-nitroanisole (PNA) and pyridine (PYR).

Results

In the dark controls and the irradiated samples with 1% solvent the pH of the samples were 6.80-7.10. In irradiated samples with 10% solvent pH was 6.7-6.9 except for vessels taken at the last sampling date, in which pH was 5.4. There were no indications of contamination that would compromise the results.

The integrated mean intensity of the artificial light penetrating the surface of the aqueous solution in the range 300 nm to 400 nm was 50.24 W/m^2. Seventeen days of exposure were calculated to be equivalent to 48.7 d and 46.7 d of natural summer sunlight at latitude 50°N and 30 to 40°N, respectively.

The loss observed in the dark controls were proposed to represent a combination of abiotic transformation and binding of tolclofos-methyl to the walls of the test vessels. The photolysis rate constant was therefore corrected:

$k_{Photolysis} = k_{Irradiated\ vessels} - k_{Dark\ control\ vessels}$. The rate constants, and the final DT_{50} and DT_{90} based on the experimental data are shown in the table below.

Table 61: Tolclofos-methyl: Rate constants (k) for irradiated samples and dark controls based on experimental data, and corrected rate constant for photolysis. DT_{50} and DT_{90} based on corrected rate constant for photolysis.

	Tolclofos-methyl (in 10% acetonitrile)	Tolclofos-methyl (in 1% acetonitrile)
$k_{Irradiated\ vessels}$, d^{-1}	0.058	0.063
$k_{Dark\ control\ vessels}$, d^{-1}	0.022	0.029
$k_{Photolysis}$, d^{-1}	0.036	0.034
DT_{50}, days	19.3	20.4
DT_{90}, days	64.0	67.7

Quantum yield for tolclofos-methyl for the different solvent concentrations were determined as follows:

$\Phi = 5.510 \times 10^{13}$ molecules / 1.490×10^{19} photons = 3.70×10^{-6} (10 % v/v acetonitrile)

$\Phi = 5.247 \times 10^{13}$ molecules / 1.503×10^{19} photons = 3.49×10^{-6} (1 % v/v acetonitrile)

Since the results were approximately identical the average quantum yield was calculated to: $\Phi = 3.6 \times 10^{-6}$.

Using the average quantum yield, the first-order rate constants (k_{pE}) and half-lives were calculated for shallow water at different seasons and latitudes of the Northern hemisphere, see Table 8.2.1.2-3.

Table 62: Tolclofos-methyl: Calculated rate constants (kpE) and DT_{50} for photolysis in shallow water at latitudes 20-50°N and different seasons, based on quantum yield for tolclofos-methyl.

Latitude	Spring	Summer	Autumn	Winter
Rate constants (k_{pE}), day^{-1}				
50°N	0.065266	0.082823	0.034407	0.014286
40°N	0.072474	0.082789	0.044932	0.029038
30°N	0.076449	0.084319	0.056082	0.042875
20°N	0.078253	0.082762	0.064926	0.054850
DT_{50}, days				
50°N	10.6	8.4	20.1	48.5
40°N	9.6	8.4	15.4	23.9
30°N	9.1	8.2	12.4	16.2
20°N	8.9	8.4	10.7	12.6

The RMS considered the study acceptable. The experimentally determined half-lives (19.3/20.4 days) are considered as more reliable than the estimate provided by Takahashi & Katagi (1988) due to the use of replicate samples and sacrifice of whole samples at analyses. Since similar results were observed for the incubations with 1 and 10% of co-solvent the RMS suggests that the overall geometric mean can be stated as endpoint: DT_{50} 19.8 days with a corresponding DT_{90} 65.9 days.

Additionally, the study provided quantum yield and calculated half-lives for shallow natural waters at different latitudes. The results obtained for 40 and 50°N are considered relevant for the EU (DT_{50}s ranging from 8.4 days in summer to 48.5 days in winter).

Conclusion

Photolysis is not an important route for the degradation of tolclofosmetyl.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable

11.2.1 Summary of data/information on environmental transformation

Not applicable

11.3 Environmental fate and other relevant information

Not applicable, see summary above.

11.4 Bioaccumulation

Since tolclofos-methyl has a log P=3.8, the potential for bioaccumulation was tested experimentally, resulting in a whole fish BCF of 670. Hence, tolclofos-methyl does fulfil the criteria for bioaccumulation.

The two major metabolites, DM-TM and ph-CH_3 had a log P of <0.3 and 2.47, repectively, and do not need to be experimentally assessed for bioaccumulative properties.

Table 63: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
ASTM E-35.21	The total ^{14}C-BCF values at 28 days of exposure for fillet, viscera and whole fish were 100, 1300, and 670, respectively.		xxxx (1986) QM-51-0019
40 CFR 158.240, OECD No. 305, FIFRA 165-4, OPPTS 850.1730	The steady state total ^{14}C-BCF for whole fish were 506 and 384 at the two tested concentrations.		xxxx (2004) QM-0059

11.4.1 Estimated bioaccumulation

Reference:	xxxx (1986) Uptake, Depuration and Bioconcentration of 14C-Rizolex by Bluegill Sunfish (*Lepomis macrochirus*)
Report No.:	QM-51-0019 33971 (994-08003)
Guideline:	ASTM E-35.21
GLP:	Yes
Previous evaluation:	In DAR (2003)
	This study was not performed in accordance with OECD TG 305. Only one concentration was tested and this was relatively high (0.030 mg/L). The calculated BCF was not corrected for lipid content. A new bioconcentration study was submitted by the applicant. This study is therefore not further evaluated. However, the applicant has provided a summary of the study which is included below.
Material and methods:	
Test item:	Radio-labelled [Phenyl-^{14}C] Tolclofos-methyl
Lot/Batch No.:	1-C-59-2
Radiochemical purity:	>99 %
Test item:	Non radio-labelled Tolclofos-methyl
Lot/Batch No.:	40810

Purity:	97.7 %
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Treatments	Nominal concentrations of 0 (acetone solvent control) and 0.030 mg/L
Number of animals	120 fish/concentration
Duration	28 days of exposure followed by 14 days of depuration
Test conditions	A flow-through system with glass aquaria containing 70 L was used. The tested concentration was 0.030 mg a.s./L and the flow rate was 350 to 420 mL/minute/aquarium resulting in replacement of the test volume 7.2 to 8.6 times per 24-hour period. The fish were acclimatized during a 14-days period to test conditions of 16 hours light and 8 hours dark, a temperature of 22 ± 2°C, pH of 8.0 – 8.2 and dissolved oxygen concentration of 7.0-8.8 mg/L. fish initial mean weight was 3.4 ± 0.92 g and initial mean standard length was 47 ± 3.7 mm. The fish were fed commercial fish food daily equivalent to 3% of their initial body weight.
Chemical analysis:	Water and fish samples were taken at 0 (immediately prior to adding the fish), 0.17, 1, 3, 7, 14, 21 and 28 days of the exposure phase and 1, 3, 7, 10, and 14 days of the depuration phase. A 500 mL sample of the test water was taken to measure the concentrations of radiocarbon and tolclofos-methyl in water. On the sampling dates, three fish were collected from both the control and treated aquaria. The control and treated fish were dissected in fillet/edible (body, muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). Three additional fish from each aquarium were used for whole fish analysis. The weights of dissected parts and whole fish were measured. In addition, supplemental fish were taken for metabolite identification on certain sampling dates. For calculation of the BCF (bioconcentration factor) and ^{14}C concentration (ppm) in fish, individual samples were homogenized with dry ice in a grinder and aliquots were subjected to combustion analysis for radioassay. The uptake rate constant (K_1) and depuration rate constant (K_2) were determined by the BIOFAC computer program. For metabolite identification, 5 g of each treated samples (21- and 28-day viscera, 21- and 28-day fillet) and 5 g of each control sample were separately homogenized with distilled water using a homogeniser. The homogenate was adjusted to pH 1 and extracted with diethyl ether. The remaining aqueous and solid fractions were refluxed for 1 hr and extracted with ether. Each extract was radioassayed. The remaining solid was combusted to measure non-extractable radioactivity. Extractable radioactive substances were identified by TLC and GC co-chromatography.

Results:

The ^{14}C-radioactivity calculated as mg/L of ^{14}C-Tolclofos-methyl in test water during the 28-day exposure period averaged 0.027 ± 0.0079 mg/L. During the depuration period, the concentration of radioactivity was negligible. Behavioural observations during the study indicated no adverse effects on control and treated fish. Two treated fish died on day 12 of the study. These deaths were considered incidental.

Under flow-through conditions at a nominal concentration of 0.030 mg/L, a steady state concentration was attained approximately 7 days after exposure. The BCF values at 28 days of exposure for fillet, viscera and whole fish were 100, 1300, and 670, respectively.

Table 64: Concentration of radioactivity (Tolclofos-methyl equivalents) in fish and BCF values calculated for fillet, viscera and whole fish (pools of 3 fish per sample) based on fresh weight

Exposure (days)	Concentration of radioactivity					
	Fillet		Viscera		Whole fish	
	ppm	BCF	ppm	BCF	ppm	BCF
Exposure						
0.17	0.94	39	4.9	200	3.1	130
1	1.3	53	14	570	7.6	310
3	1.8	69	28	1100	16	610
7	3.2	120	30	1100	20	750
14	1.1	45	25	1000	12	490
21	2.8	110	31	1200	20	770
28	2.7	100	36	1300	18	670
Depuration						
1	0.87		25		13	
3	0.14		0.90		0.93	
7	0.058		0.28		0.22	
10	0.057		0.28		0.15	
14	0.043		0.28		0.18	

The clearance times (CT_{50}, CT_{90} and CT_{95}) were also calculated using a non-linear two-compartment kinetic modelling computer program (BIOFAC). The results of these calculations are shown in Table 8.2.2.3 2. The uptake rate Constant K1, and the clearance rate constant K_2, for whole fish were 410 day^{-1} and 0.63 day^{-1}, respectively.

Table 65: The clearance time (CT_{50}, CT_{90}, CT_{95}) of radioactivity in fish during depuration phase

	Clearance time (days)		
	Fillet	Viscera	Whole fish
CT_{50}	0.66	1.1	1.1
CT_{90}	2.2	3.7	3.6
CT_{95}	2.9	4.8	4.7

The distribution of metabolites in fish is shown in the table below. The majority of the accumulated radioactivity was Tolclofos-methyl in both fillet (79 to 81 %) and viscera (39 to 47 %). Of the metabolites TMO and ph-COOH (fillet) and TM-CHO, ph-COOH, TMO and ph-CH_2OH (viscera) were detected at

levels greater than 5 % of total radioactive residue in tissue. Additionally, one unknown metabolite was detected at levels of approximately 6 % in viscera. Tolclofos-methyl in fish was metabolized by oxidation of the para-methyl group (35 – 39 % of total radiocarbon in the viscera and 4 to 8 % in the fillet).

Another pathway was via cleavage of the P-O-acryl linkage to form phenolic metabolites (4 to 8 % in the fillet and 12 to 27 % in the viscera). Of the total radiocarbon in tissue, the desulfuration reaction and the formation of an oxon analogue accounted for 4 to 5 % in the viscera and 1 to 9 % in the fillet.

Table 66: Metabolites and percentage of total radioactivity in the fillet and viscera

	Percentage of total radioactive residue in tissue			
	Fillet		Viscera	
	Day 21	Day 28	Day 21	Day 28
TM (parent compound)	79.14	91.17	45.85	38.79
TM-CHO	0.00	0.00	22.37	12.30
ph-CHO	0.00	1.35	2.78	2.23
ph-COOH	6.66	1.95	3.81	18.76
TMO	9.32	1.01	4.06	5.20
Ph-CH_2OH	1.82	0.83	5.84	6.16
Unknown-1	0.42	1.54	6.07	5.51
Unknown-2	0.64	0.97	0.43	0.84
Unknown-3	1.82	2.15	0.29	0.94
Unknown-4	0.00	0.00	0.32	1.00
Unknown-5	1.35	0.71	2.81	3.65
Unknown-6	2.33	1.89	2.16	3.13
Unknown-7	1.14	2.62	1.99	1.92
Unextractable	5.01	7.25	6.40	5.60

The chemical name and abbreviation of the metabolites are shown below.

Table 67: Identity of metabolites

Designation	Chemical name
TM (parent compound)	O,O-dimethyl o (2,6-dichloro-4-methylphenyl) phosphorothioate
TMO	O,O-dimethyl o (2,6-dichloro-4-methylphenyl) phosphate
TMO-CHO	O,O-dimethyl o (2,6-dichloro-4-formylphenyl) phosphorothioate
ph-CHO	3,5-dichloro-4-hydroxybenzaldehyde
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid
ph-CH_2OH	3,5-dichloro-4-hydroxyenzyl alcohol

Under flow-through conditions at a nominal concentration of 0.030 mg a.s./L, a steady state concentration was attained approximately 7 days after exposure. The BCF values at 28 days of exposure for fillet, viscera and whole fish were 100, 1300, and 670, respectively.

Conclusion

The resulting BCF values from this study was slightly higher than the corresponding total-C^{14}-BCF values from the more recent study by xxxx (2004) summarised below. The applicant has provided the following justification for selection of BCF value for the risk assessment and therefor also for the CLH report of tolclofos-methyl:

"The study by xxxx (1986) is considered to have several shortcomings (only one concentration tested, the tested concentration was not 1 % of the LC_{50} and no measurements of TOC were performed). Thus the study was accepted only as indication of the BCF. Therefore the more recent bioconcentration study by xxxx (2004) is considered to be the relevant study for derivation of the BCF, even if the estimated BCF in the study by xxxx (1986) was higher.

For the secondary poisoning risk assessment (fish-eating birds and mammals) the steady-state BCF of 131 from the study by xxxx (2004) calculated for the high test item treatment group was used.

According to the RMS, no clear steady state was reached for the high concentration. For the low concentration, a steady state was reached on day 14. The RMS agreed that the BCFk and the BCFss are not significantly different but used the highest lipid corrected BCFk of 144 for the risk assessment as it is independent of reaching a steady state."

The RMS acknowledges the shortcomings of this study. It is noted though that the total-C^{14}-BCF whole fish value in this study (BCF=670 on day 28, which is also equal to the mean value from steady state at 7 days until the end of the study) is similar to the steady state value obtained from the lower test concentration in the more recent study presented below (total-C^{14}-BCF=506). Since no information is available on the toxicity of the metabolites observed in the BCF studies, the total-C^{14}-BCF values are the most appropriate for the risk assessment. Further, the risk assessment should be based on the BCF without lipid correction (the lipid corrected values are only used for the PBT-assessment.

Overall from this study it can be concluded that tolklofos-methyl BCF in fish=670, which triggers the CLP criteria BCF for fish >500 and has therefor a high bioaccumulation potential.

Reference:	xxxx (2004) Bioconcentration of [^{14}C]Tolclofos-methyl by Bluegill Sunfish (*Lepomis macrochirus*)
Company Report No.:	QM-0059
	016416-1 (872-001)
Guideline:	40 CFR 158.240, OECD No. 305, FIFRA 165-4, OPPTS 850.1730
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	Radio-labelled [^{14}C] Tolclofos-methyl

	Tolclofos-methyl (not radiolabelled)
Lot/Batch No:	RIS2003-011 (radiolabelled)
	000427G
Purity:	>99% (radiolabelled)
	99.6%
Species:	Bluegill sunfish *Lepomis macrochirus* (juvenile)
Test media:	Well water, characterized as soft (36-40 mg CaCO3/L). Flow rate: 8.0 tank volume replacements/day
Treatments:	Control (acetone 0.05 mL/L), 0.001 mg/L and 0.010 mg/L (highest concentration corresponding to ~1/100 of the 96-hours LC_{50} >720 µg/L)
Duration:	35 days exposure phase and 14 days depuration phase.
Exposure:	Exposure took place after 14 days of acclimation. The test was performed in a flow-through system in 73L glass aquaria with 150 fish/aquarium, corresponding to a biomass of 270 g.
	The fish were fed commercial available flaked food daily, *ad libitum*.
Test conditions:	Photoperiod: 16:8 light:dark
	Temperature: 23±2°C
	pH: 6.9-7.5 (exposure phase), 7.2-7.8 (depuration phase)
	Oxygen level: 6.8-7.5 mg/L (exposure phase), 7.5-8.8 mg/L (depuration phase)
	TOC: 13.0-22.5 mg/L (exposure phase). 0.53-0.71 mg/L (depuration phase)
Observations:	10 fish from each control and treatment were removed from the aquarium and analysed on day 1, 3, 7, 14, 21, 28 and 35 (exposure phase) and on day 1, 3, 7 and 14 (depuration phase). Wet weight of the sacrificed fish were recorded at each sampling time.
	Observations were made daily for appearance, behaviour and mortality.
Chemical analysis:	Water samples were taken at day -2, -1, 0, 1, 3, 7, 14, 21, 28 and 35 during exposure phase and at day 1, 3, 7 and 14 during depuration phase. Liquid Scintillation Counting (LSC) was used to monitor the concentration of [^{14}C] residues in the exposure water (LOQ = 0.0000127 mg/L). Water sampled during exposure phase was also analysed by HPLC-RAM (LOQ = 0.0000153 mg/L).
	Samples for metabolite characterization were taken at day 14 and 28 during exposure phase.
	Tissue samples from the sacrificed fish were extracted and analysed for lipid content, TRR by LSC and distribution of tolclofos-methyl and metabolites by HPLC-RAM.
Data analysis:	BCF_{ss} values were calculated by dividing the mean measured total ^{14}C-concentrations in the edible and non-edible portions and in the whole fish with the mean measured total ^{14}C-concentrations in the water phase at

steady state (day 21-35). BCF_{ss} was divided with lipid content to correct for lipids.

BCF_k was calculated by dividing uptake rate constant (K_u) with depuration rate constant (K_d).

Results:

Observations of Mortality and Clinical Signs of Toxicity:

No mortalities occurred in the population 48 hour prior to the test initiation. One mortality each occurred in the solvent control and in the 0.001 mg/L treatment. In the 0.01 mg/l treatment, three mortalities occurred, including one accidental. Otherwise, the fish appeared healthy and exhibited normal behaviour throughout the study.

Test Concentrations in Water:

During the exposure phase, LCS results determined that the mean measured concentrations for total radioactivity per sampling interval ranged from 96-110 % of nominal in the 0.001 mg a.s./L treatment and from 10-120 % of nominal in the 0.01 mg a.s./L treatment. No [^{14}C] residues were detected in the solvent control or in the depuration water after day 1. Analysis of the quality control samples gave recoveries ranging from 99.6 to 112 %.

The measured concentration of [^{14}C]Tolclofos-methyl, analysed with HPLC/RAM, in the exposure water ranged from 65-93% and 69-105% of the nominal in the 0.001 mg/l and 0.010 mg/L treatments, respectively. During depuration phase, [^{14}C]Tolclofos-methyl concentrations were below LOQ. Mean percent recovery of the quality control was 85.9-118%.

Concentrations in Fish Tissues:

The mean tolclofos-methyl concentration in the 0.001mg/L treatment during the exposure phase were 0.0390, 0.129 and 0.0794 mg/kg for the edible, non-eatable portions and in the whole fish, respectively. The high concentration treatment resulted in mean ^{14}C concentrations in fish of 0.429, 1.34, 0.840 mg/kg in the eatable, non-eatable portions and in the whole fish, respectively.

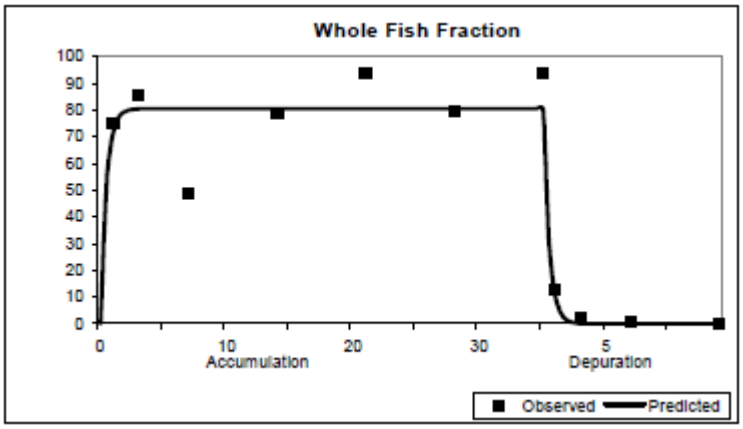


Figure 9.1.3-1. Concentration of tolclofos-methyl in whole fish over time from exposure to 0.001 mg/L and depuration phase.

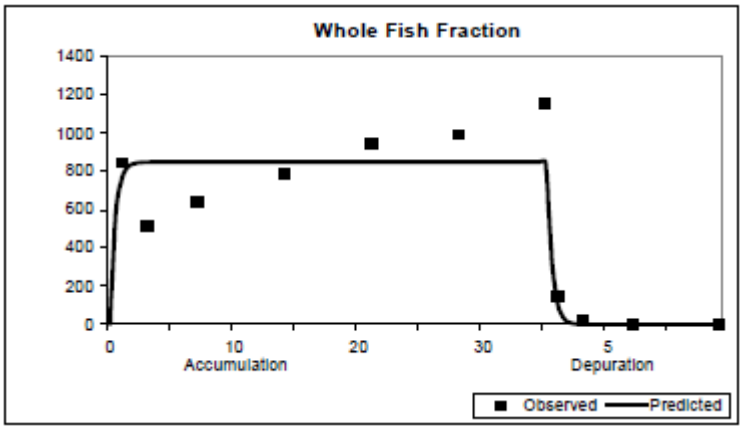


Figure 9.1.3-2. Concentration of tolclofos-methyl in whole fish over time from exposure to 0.010 mg/L and depuration phase.

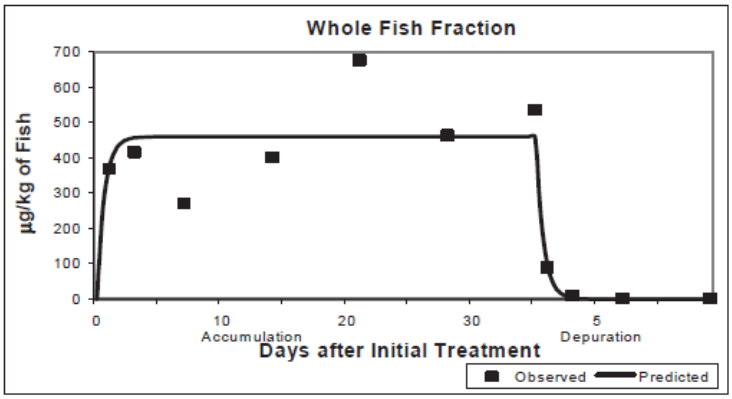


Figure 9.1.3-3. Concentration of total radioactive residue (TRR) in whole fish over time from exposure to 0.001 mg/L and depuration phase.

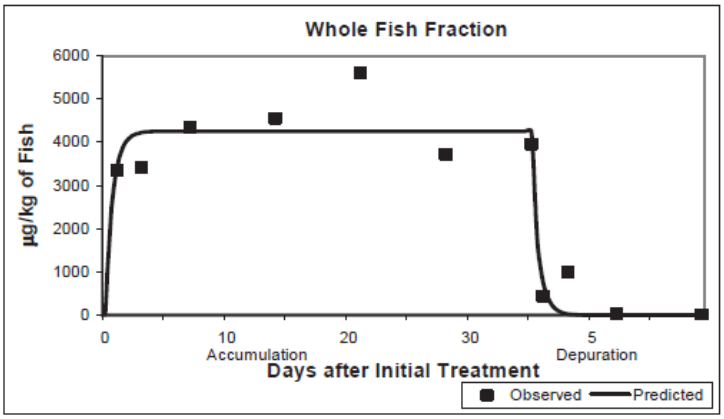


Figure 9.1.3-4. Concentration of total radioactive residue (TRR) in whole fish over time from exposure to 0.010 mg/L and depuration phase.

There was a rapid decline in ^{14}C concentration and concentration of tolclofos-methyl in the fish tissue during depuration phase. The ^{14}C concentration for whole fish ranged from 0.0133-0.0002 mg/kg during the 14-day depuration phase for the lowest concentration and 0.147-0.0013 mg/kg for the highest concentration.

Lipid analysis:

The average lipid content in whole fish was 4.32 and 4.54% for the low and high concentrations, respectively.

Quantification and Identification of Metabolites:

Tolclofos-methyl was the major residue found in the exposure water. DCMP and ph-CH_2OH were found in minor parts.

Table 68: Distribution of ^{14}C-residues in exposure water extract from the high concentration test.

	Day 14 % Distribution	Day 28 % Distribution
Tolclofos-methyl	94.65	85.57
DCMP	5.35	12.54
ph-CH_2OH	---	1.90

Tolclofos-methyl was extensively degraded in fish from the beginning of the exposure phase and concentrations ranged 13.8-29.3% TRR in the whole fish. The major metabolites detected from the fish tissue throughout the study were DCMP (3.4-14.8 % TRR) and DM-TM-CH_2OH (9.7-22.5 % TRR). A broad region of radioactivity eluted between 38-44 minutes in the methanol:water fraction using HPLC. This region contained 21.7-58.9% TRR in the whole fish. DCMP was the major component present in this fraction.

Table 69: Summary of rate constants and bioconcentration factors

Exposure concentration (mg a.s./L)		ku	kd	BCFk	BCFSS		Lipid corrected BCFSS
TRR[1)]							
0.001	Whole fish	699	1.68	417	506		117
0.010	Whole fish	614	1.66	369	384		85
Tolclofos-methyl							
0.001	Whole fish	220	2.22	99	110		25
0.010	Whole fish	263	2.44	108	131		29

[1)]Total Radioactive Residue

Conclusion

This study fulfils the validity criteria according to OECD TG 305 (2012) with the exception of a deviating water concentration at day 0. This initially higher water concentration was transient and from day1, the concentration of tolclofos-methyl in the test water was within ±20% of the mean measured.

The highest tested concentration (0.010 mg/L) is very close to NOEC for early stage toxicity for fish (0.012 mg/l). However, this value was set for rainbow trout which, based on available acute toxicity data, was more sensitive than bluegill which was used in this study.

The calculated BCFSS were normalized to the mean lipid content during exposure phase. However, according to OECD TG 305 (2012), lipid corrected BCF should be calculated for a lipid content of 5%. BCF corrected for lipid content according to the calculation in OECD TG 305 (2012) are presented in the table below.

Table 70: Summary of bioconcentration factors

Exposure concentration (mg a.s./L)		BCF_k	BCF_{SS}	Mean lipid content during exposure phase (%)	Mean lipid content at the end of exposure phase (%)	Average lipid content (%) according to OECD TG 305	Lipid corrected BCFk (5% lipids)	Lipid corrected BCFSS (5% lipids)
TRR[1)]								
0.001	Whole fish	417	506	3.92	4.60	5	532	550
0.010	Whole fish	369	384	3.76	4.74	5	491	405
Tolclofos-methyl								
0.001	Whole fish	99	110	3.92	4.60	5	126	120
0.010	Whole fish	108	131	3.76	4.74	5	144	138

[1)]Total Radioactive Residue

No clear steady state was reached for the highest concentration of tolclofos-methyl or total radioactive residue in fish tissue during the exposure phase (see figure 9.2.2-2-4) with the exception of the concentration of tolclofos-methyl in the fish exposed to 0.001 mg/L. At this concentration, steady state was reached on day 14. The BCF_k and BCF_{ss} are not significantly different from each other when based on tolclofos-methyl concentrations. However, the lipid corrected BCFk based on calculations from the highest exposure level and concentration measured with HPLC will be used in the risk assessment as it is independent of reaching steady state.

No confidence intervals were reported for the BCF-values.

11.5 Acute aquatic hazard

Table 71: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
FIFRA Guideline 72-1, OPPTS 850.1075, OECD No. 203, EC Guideline Annex V - Method C.1.	*Oncorhynchus mykiss* Rainbow Trout	Tolclofos-methyl	Acute 96 hr (flow-through), LC_{50}=0.69 mg a.s./L (mm)	Key study Reliability 1	xxxx (2003) QW-0071
FIFRA Guideline 72-1, OPPTS 850.1075, OECD No.	*Lepomis macrochirus* Bluegill	Tolclofos-methyl	Acute 96 hr (flow-through), LC_{50}>0.720 mg a.s./L (mm)	Reliability 1	xxxx (1989) QW-91-0036

203, EC Guideline Annex V - Method C.1.					
FIFRA Guideline 72-1, OPPTS 850.1075, OECD No. 203, EC Guideline Annex V - Method C.1.	*Oncorhynchus mykiss* Rainbow Trout	DM-TM	Acute 96 hr (static), LC_{50}=110 mg metabolite/L (mm)	Reliability 1	xxxx (1998a) QW-0055
EPA FIFRA, 40 CFR, Part 158.145, Guideline 72-2	*Daphnia magna* Water flea	Tolclofos-methyl	Acute 48 h (static) 48 mg a.s./L (mm)	Reliability 1	Murrell, H. et al. (1994) QW-41-0046
OECD TG 202 (1984)	*Daphnia magna* Water flea	DM-TM	Acute 48 h (static) >95 mg metabolite/L (mm)	Reliability 1	Dionne, E. (1998b) QW-0054
OPPTS 850.1035 U.S EPA Guideline	*Americamysis bahia* Saltwater mysid	Tolclofos-methyl	96 h (semi-static LC_{50})= 0.377 mg a.s./L (mm)	Key study Reliability 1	Palmer, S.J. *et .al* (2010a) QW-0111
OPPTS 850.1035 U.S EPA Guideline	*Americamysis bahia* Saltwater mysid	DM-TM	96 h (static LC_{50})= >100 mg metabolite/L (mm)	Reliability 1	Shaw, A.C. (2014a) QW-0147
OPPTS 850.1035 U.S EPA Guideline	*Americamysis bahia* Saltwater mysid	Metabolite ph-CH_3	96 h LC_{50}=1.1 metabolite mg/L	Reliability 1	Shaw, A.C. (2014b)
OECD No. 201, EC Guideline Annex V - Method C.3. OECD 201 (2011) (for the re-calculations)	*Scenedesmus subspicatus*	Tolclofos-methyl	72 h (static) EyC_{50}=0.49 mg a.s. /L EC_{50} (growth) no information) NOEC =0.12 mg a.s/L	Key study Reliability 1	Sayers, L.E. (2003) QW-0072 and Wirzinger,G *et al.* (2014) QW-0144
OECD No. 201, EC Guideline Annex V - Method C.3. OECD 201 (2011) (for	*Scenedesmus subspicatus*	DM-TM	48 h (static) EC_{50} (growth) >97 mg a.s./L (water solubility limit)	Reliability 1	Hoberg, J.R. (1998) QW-0053; Wirzinger & Ruhnke (2016), QW-0157

the re-calculations)					

[1] Indicate if the results are based on the measured or on the nominal concentration

11.5.1 Acute (short-term) toxicity to fish

Reference:	xxxx (2003) Tolclofos-methyl – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Flow-through Conditions
Company Report No.:	QW-0071 13048.6416 (994-08024)
Guideline:	FIFRA Guideline 72-1, OPPTS 850.1075, OECD No. 203, EC Guideline Annex V - Method C.1.
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	00668G
Purity:	97.8%
Species:	Rainbow Trout (*Oncorhynchus mykiss*)
Test system/test conditions:	Juvenile fish (length: 46-58 mm, weight: 0.80-1.9 g) were exposed to tolclofos-methyl for 96 hours in a flow-through system with two replicates per treatment and control with 10 animals each (20 animals for each treatment and control). The loading rate was 0.36 g of fish/L of solution per day. Tested concentrations were control, solvent control, 0.21, 0.36, 0.59, 0.99, 1.6 mg/L. The test substance was dissolved with acetone and diluted with the dilution water having a pH of 7.8. The concentrations in the test solutions were analysed using HPLC/UV at 0 and 96 hours. All test solution samples were centrifuged prior to extraction and analysis. The LOD was 0.00981 mg/L and the LOQ was 0.05 mg/L. The test conditions were (range of daily measurements): temperature of 11-13°C; pH 6.5-7.6; dissolved oxygen: 6.5-9.1 mg/L (60-84% saturation). Mortality and sublethal responses were observed after 0, 3, 6, 24, 48, 72 and 96 hours of exposure. LC_{50} was calculated by probit analysis.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean measured concentrations were determined to be 0.17, 0.25, 0.35, 0.61, and 0.80 mg a.s./L, representing 82, 69, 59, 62, and 50 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 72: Measured Tolclofos-methyl concentrations in the exposure solutions during the 96-hour exposure of *Oncorhynchus mykiss*

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L] [a]			Cumulative mortality (n=20)
	0 hour	96 hour	Mean concentration	96 hours

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L] [a]			Cumulative mortality (n=20)
	0 hour	96 hour	Mean concentration	96 hours
0 (control)	< LOQ	< LOQ	-	0 : 20N
0 (Solvent control)	< LOQ	< LOQ	-	0 : 20N
0.21	0.21	0.14	0.17 (82)	0 : 20N
0.36	0.25	0.24	0.25 (69)	0 : 20N
0.59	0.31	0.38	0.35 (59)	0 : 20N
0.99	0.64	0.58	0.61 (62)	4 : 1L, 2PLE, 13CLE
1.6	0.91	0.69	0.80 (50)	17 : 3CLE

[a] values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 0.024 mg a.s./L

N= Normal; L=Lethargic; PLC=Partial Loss of Equilibrium; CLE=Complete Loss of Equilibrium

Conclusion

Based on the results of this study the acute toxicity (96-hour LC_{50}) of tolclofos-methyl to rainbow trout was 0.69 mg/L, with 95% confidence intervals of 0.64 – 0.74 mg/L. NOEC was 0.35 mg/L.

It therefor can be concluded that tolclofos-methyl is very toxic to fish with LC_{50}< 1 mg/L with the most sensitive species rainbow trout *Oncorhynchus mykiss* with 96 h LC_{50} = 0.64 mg/L.

Reference:	**xxxx (1989)** Acute Flow-through Toxicity of Rizolex® Technical to Bluegill (*Lepomis macrochirus*)
Company Report No.:	QW-91-0036 37795 (994-08018)
Guideline:	U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-1
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	40810
Purity:	97.2%
Species:	Bluegill (*Lepomis macrochirus*)
Test system/test conditions:	Juvenile fish (length: 4.8±0.39 cm) were exposed to tolclofos-methyl for 96 hours in a flow-through system with 20 animals for each treatment and control. Tested concentrations were control, solvent control, 0.046, 0.093, 0.190, 0.370 and 0.740 mg/L. The test substance was dissolved with acetone and diluted with the dilution water. The concentrations in the test solutions were analysed using GC-ECD (LOQ = 2.2 µg/L) at 0 and 96 hours. All test solution samples were centrifuged prior to extraction and analysis. The test conditions were (range of daily measurements): temperature of 21-22°C; pH 7.7-8.0; dissolved oxygen: 7.3-7.9 mg/L (87-94% saturation). Mortality and sublethal responses were observed once every 24 hours. LC_{50} was calculated by binomial, moving average and probit analysis.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean measured concentrations were determined to be 0.043, 0.093, 0.179, 0.360, and 0.720 mg a.s./L, representing 93, 100, 97, 97, and 97 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 73: Measured Tolclofos-methyl concentrations in the exposure solutions during the 96-hour exposure of Lepomis macrochirus

Nominal concentration [mg a.s./L]	**Measured concentration [mg a.s./L] a**		
	0 hour	96 hour	Mean concentration
0 (control)	< LOQ	< LOQ	-
0 (Solvent control)	< LOQ	< LOQ	-
0.046	0.044	0.042	0.043 (93)
0.093	0.094	0.091	0.093 (100)
0.185	0.186	0.172	0.179 (97)
0.370	0.370	0.350	0.360 (97)
0.740	0.750	0.690	0.720 (97)

a values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 0.0022 mg a.s./L

No mortality or behaviour effects occurred after 96 hours exposure. LC_{50} was therefore set to above highest measured concentration (>720 µg a.i./L).

RMS comments and conclusion:

The highest tested concentration corresponded to the compound solubility limit and the maximum solvent concentration was used (0.10 mL acetone/L). The study and LC_{50} >720 µg a.i./L is therefore accepted. The length of the fish was higher in the reported study (4.8±0.39 cm) than suggested by the OECD TG 203 (1992) (2.0±1.0 cm). This may have had an effect on the results as larger fish generally are less sensitive. The study fulfils the validity criteria of OECD TG 203 (1992).

Reference:	**xxxx (1998a)** Desmethyl-tolclofosmethyl - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Static Acute Conditions
Company Report No.:	QW-0055 13048.1198.6177.103 (994-08028)
Guideline:	OECD 203
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	DM-TM
Lot/Batch No:	CTS98009G
Purity:	98.9%
Species:	Rainbow Trout (*Oncorhynchus mykiss*)
Test system/test conditions:	Juvenile fish (length: 40-58 mm, weight: 0.68-1.8 g) were exposed DM-TM for 96 hours under static test conditions with 10 animals for each treatment and control (0.8 g biomass/L test solution).

Tested concentrations were control, 18, 30, 50, 84 and 140 mg/L. The concentrations in the test solutions were analysed using HPLC/UV at 0 and 96 hours (LOQ = 0.108 mg a.s./L). All test solution samples were centrifuged prior to extraction and analysis.

The test conditions were (range of daily measurements): temperature of 14-15°C; pH 6.8-7.3; dissolved oxygen: 6.2-9.4 mg/L (60-91% saturation).

Mortality and sublethal responses were observed after 0, 3, 6, 24, 48, 72 and 96 hours exposure. LC_{50} was empirically tested.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean measured concentrations were determined to be 14, 24, 39, 64, and 110 mg a.s./L, representing 78, 79, 77, 77, and 78 % of the nominal concentrations, respectively.

Table 74: Measured DM-TM concentrations in the exposure solutions during the 96-hour exposure of Oncorhyncus mykiss

Nominal concentration [mg/L]	Measured concentration [mg/L] a			
	0 hour (new)	96 hour (spent)	Arithmetic mean concentration	Geometric mean concentration
0 (control)	< LOQ	< LOQ	-	-
18	14	14	14 (78)	14 (78)
30	24	24	24 (79)	24 (79)
50	38	39	39 (77)	39 (77)
84	64	64	64 (77)	64 (77)
140	110	110	110 (78)	110 (78)

a values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 1.3 mg/L

The results of the study were based on the geometric mean measured concentrations. A 96 h LC_{50} of > 110 mg DM-TM/L was derived. No mortality or behaviour effects occurred after 96 hours exposure.

RMS comments and conclusion:

This study is well performed and reported. The study fulfils the validity criteria of OECD TG 203 (1992). Although the arithmetic mean of the measured concentrations was calculated this had no effect of the effect level as the concentrations was constant throughout the exposure.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Reference:	**Murrell, H, Bucksath, J.D. (1994)** Acute toxicity of Rizolex to *Daphnia magna*
Company Report No.:	QW-41-0046 41242 (994-08021)
Guideline:	EPA FIFRA, 40 CFR, Part 158.145, Guideline 72-2
GLP:	Yes

Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	30909G
Purity:	98.8%
Species:	*Daphnia magna*
Test system/test conditions:	The test was performed in a static system with 250 mL glass beakers containing 200 mL test volume at a 16:8 h light:dark photoperiod. Tested concentrations were control, solvent control, 15, 24, 38, 62 and 100 mg/L. Each test concentration had two replicates with 10 animals each (age <24h). Temperature was 20-21°C, pH 7.9-8.1 and dissolved oxygen 6.3-8.4 mg/L. Immobility and abnormal behaviour were observed after 24 and 48 hours. Water concentrations were measured using GLC (LOQ <0.808 mg/mL). EC_{50} was calculated using the binomial, moving average and probit test.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean *measured* concentrations were determined to be 8.4, 17, 26, 41, and 64 mg a.s./L, representing 56, 71, 68, 66, and 64% of the nominal concentrations, respectively.

Table 75: Measured Tolclofos-methyl concentrations in the exposure solutions during the 48-hour exposure of Daphnia magna

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L] a			
	0 hour (new)	48 hour (spent)	Arithm. mean concentration	Geomean concentration
0 (control)	< LOQ	< LOQ	-	-
0 (solvent control)	< LOQ	< LOQ	-	-
15	8.29	8.4	8.4 (56)	8.35 (55.6)
24	16.0	16.0	17 (71)	17 (71)
38	23.4	28.3	26 (68)	25.7 (67.7)
62	43.4	38.3	41 (66)	40.8 (65.8)
100	62.7	65.7	64 (64)	64.2 (64.2)

[a] values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 0.808 mg a.s./L

In the study report, the results of the study were based on the arithmetic mean measured concentrations but were re-calculated by Wirzinger et al. (2017a). Based on the results of this study the acute toxicity (48-hour EC_{50}) of tolclofos-methyl to *Daphnia magna* was 48 (95%CL 43 – 53) mg/L.

Table 76. Immobility and behavioural observations during the acute toxicity test of tolclofos-methyl to Daphnia magna

Nominal concentrations (mg/L)	Mean measured concentration (mg/L)	Cumulative immobility (n=20)	
		24 hours	48 hours
Control	NA	0 : 20 Normal	0 : 20 Normal
0	NA	0 : 20 Normal	0 : 20 Normal
15	8.4	0 : 20 Normal	0 : 20 Normal
24	17	0 : 20 Normal	0 : 20 Normal
38	26	0 : 20 Normal	0 : 7 Normal, 13 On bottom
62	41	0 : 13 Normal, 7 On bottom	5 : 2 Normal, 13 On bottom
100	64	0 : 15 Normal, 5 On bottom	18 : 2 On bottom

Precipitations and cloudy solutions were observed for all tested concentrations within 48 hours. During EU review further information on the presence of undissolved test substance and cloudy solutions was requested. Precipitation and cloudy solutions were observed for all tested concentrations. During the test, daphnids were observed on the bottom of the vessels at measured concentrations ≥ 26 mg a.s./L. However immobile daphnids were only observed at ≥ 41 mg a.s./L. The NOEC based on lack of immobility and abnormal effects was determined to be 17 mg a.s./L. Thus it is considered that the presence of undissolved test substance would not have an impact on the results of the test.

RMS comments and conclusion:

This study uses fewer replicates than recommended by the OECD TG 202 (2004) (recommends 4 replicates with 5 animals each). However, since the EC_{50} is calculated based on total number of daphnids, the number of replicates is unrelated to 95% confidence intervals. Therefore, the low number of replicates is not considered to have a major influence on the results. Although arithmetic mean instead of geometric mean was used to calculate mean measured concentrations, this had no impact on the effect value.

The RMS agrees with the conclusions made by the applicant and the study is considered acceptable.

Reference:	**Dionne, E (1998b)** Desmethyl-Tolclofosmethyl – Acute Toxicity to Daphnids (*Daphnia magna*) Under Static Conditions
Company Report No.:	QW-0054 13048.1198.6178.110 (994-08027)
Guideline:	OECD TG 202 (1984)
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	DM-TM
Lot/Batch No:	CTS98009G
Purity:	98.9%
Species:	*Daphnia magna*
Test system/test conditions:	The test was performed in a static system with 250 mL glass beakers containing 200 mL test volume at a 16:8 h light:dark photoperiod. Tested concentrations were control, 16, 27, 45, 75 and 125 mg/L. Each test concentration had 4 replicates with 5 animals each (age <24 h). Temperature was 20-21°C, pH 8.0-8.2 and dissolved oxygen 8.1-9.5 mg/L.

Immobility and abnormal behaviour were observed after 24 and 48 hours.

Water concentrations were measured using HPLC/UV (LOQ = 0.108 mg a.s./L).
LC_{50} and NOEC were determined empirically.

Results:

The results of the chemical analysis for DM-TM are presented in the table below. The arithmetic mean of the measured concentrations was calculated and presented in the study report. The arithmetic mean measured concentrations were determined to be 12, 20, 34, 50, and 95 mg/L, representing 74, 73, 75, 67, and 76 % of the nominal concentrations, respectively.

The measured concentrations in the study were constant throughout the study with exception of the nominal 27 mg/L treatment group were the 0 hour measured test item concentration was 20 mg/L and the measured value at test end was 19 mg/L and in the nominal 125 mg/L treatment group were the 0 hour measured test item concentration was 96 mg/L and the measured value at test end was 93 mg/L. Thus, both arithmetic and geometric calculation end up in identical values (19.5 mg/L and 19.49 mg/L, and 94.5 mg/L and 94.49 mg/L respectively). Therefore, this had no impact on the endpoint. Thus it is considered that the arithmetic mean measured concentrations are also appropriate. Nevertheless, a 48 h EC_{50} of > 95 mg/L was derived based on geometric mean measured concentrations.

Table 77: Measured DM-TM concentrations in the exposure solutions during the 48-hour exposure of Daphnia magna

Nominal concentration [mg/L]	Measured concentration [mg/L] a			
	0 hour (new)	48 hour (spent)	Arithm. mean concentration	Geomean concentration
0 (control)	< 1.1	< 1.0	-	-
16	12	12	12 (74)	12 (74)
27	20	19	20 (73)	20 (72)
45	34	34	34 (75)	34 (75)
75	50	50	50 (67)	50 (67)
125	96	93	95 (76)	95 (76)

[a] values in parentheses represent % of nominal concentration

n.d. not determined

No effects on immobility or behaviour due to toxicity of DM-TM were observed and it was therefore concluded that LC_{50} >95 mg/L and NOEC was set to the highest concentration (95 mg/L based on the arithmetic mean measured test concentrations).

Table 78. Immobility and behavioural observations during the acute toxicity test of DM-TM to Daphnia magna

Nominal concentrations (mg/L)	Mean measured concentration (mg/L)	Cumulative immobility (n=20)	
		24 hours	48 hours
Control	0	0 : 20 Normal	0 : 20 Normal
16	12	0 : 20 Normal	0 : 20 Normal
27	20	0 : 20 Normal	0 : 20 Normal
45	34	0 : 20 Normal	0 : 20 Normal
75	50	0 : 20 Normal	0 : 20 Normal
125	95	0 : 20 Normal	0 : 20 Normal

RMS comments and conclusion:

This study is well performed and reported. All validity criteria according to OECD 202 (2004) were fulfilled and the study is therefore accepted. Although arithmetic mean instead of geometric mean was used to calculate mean measured concentrations, this had little impact on the effect value.

Reference:	Palmer, S.J., Schneider, S.Z., Kendall, T.Z., Krueger, H.O. (2010a) V-10178 - A 96-Hour Static-renewal Acute Toxicity Test with the Saltwater Mysid (*Americamysis bahia*)
Company Report No.:	QW-0111
	263A-112
	VP-37053(825-002)
Guideline:	OPPTS 850.1035
	U.S EPA Guideline
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	Tolclofos-methyl Technical grade (V-10178)
Lot/Batch No:	AS 2218c
Purity:	95.6 %
Species:	Saltwater mysids (*Americamysis bahia*)
Test media:	Natural seawater filtered through sand filter and diluted to a salinity of approx. 20‰. The water was then filtered through 0.45 µm and passed through an ultraviolet (UV) sterilizer.
Treatments:	Nominal: 0 (control), 0 (solvent control), 63, 125, 250, 500 and 1000 µg a.i./L
	Arithmetic mean measured: <LOQ, <LOQ, 60, 121, 230, 458, 742 µg a.i./L
Controls:	Negative control and solvent control (0.1 mL/L dimethylformamide)
Duration:	96 h
Exposure:	Each test vessel (glass beaker; 2 L) served as one replicate and was filled with 1.5 L of test water. Each control and treatment had two replicates with 10 animals (total 20 animals/treatment). The animals were <24h old at the start of the test.
Test conditions:	Photoperiod 16:8 light:dark
	Temperature: 25±2°C

	pH: 8.1-8.3
	Dissolved oxygen: 6.3-7.2 mg/L
Observations:	Mortality and abnormal behaviour were evaluated after 4.5, 24, 48, 72 and 96 hours.
Chemical analysis:	Chemical analyses of V-10178 were performed using an Agilent Model 5890 Gas Chromatograph (GC) with an Electronic Capture Detector (GC/ECD). LOQ was 30 µg a.i/L. Samples were taken from both newly prepared test solution and 24-hour old test solution (2 replicates for old test solution).
Data analysis:	Probit analysis. Nonlinear interpolation to calculate EC_{50} (48, 72, 96 h). LC_{50} (24 h) and NOEC were determined by visual interpretation due to mortality <50% after 24 hours.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The arithmetic mean measured concentrations were determined to be 60, 121, 230, 458 and 742 µg a.s./L, representing 95, 97, 92, 92, and 74 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 79: Measured Tolclofos-methyl concentrations in the exposure solutions during the 96 hour test with *Americamysis bahia*

Nominal concentration [mg a.s./L]	Measured concentration [mg a.s./L] [a]				
	0 hours (new)	24 hours (spent)	72 hours (new)	96 hours (spent)	Mean concentration
0 (control)	< LOQ	< LOQ	< LOQ	< LOQ	-
0 (solvent control)	< LOQ	< LOQ	< LOQ	< LOQ	-
0.063	0.062 (98.5)	0.059 (93.3)	0.062 (98.3)	0.059 (93.1)	0.060 (95)
0.125	0.125 (99.9)	0.118 (94.2)	0.122 (97.4)	0.119 (94.7)	0.121 (97)
0.250	0.240 (96.0)	0.213 (85.0)	0.240 (96.2)	0.225 (89.9)	0.230 (92)
0.500	0.471 (94.2)	0.448 (89.5)	0.473 (94.5)	0.439 (87.8)	0.458 (92)
1.00	0.765 (76.5)	0.699 (69.9)	0.762 (76.2)	- [b]	0.742 (74)

[a] values in parentheses represent % of nominal concentration

[b] samples not taken due to 100 % mortality at 72 h

measured concentrations for spent solutions based on average of two replicates)

n.d. not determined

LOQ = 0.030 mg a.s./L

The NOEC was determined to be 121 µg a.s./L, based on the arithmetic mean of the measured test concentrations. EC_{50} values at 24, 48, 72 and 96 hours were determined from the mortality data based on measured concentrations.

Table 80: Summary of effects in saltwater mysids (*Americamysis bahia*) exposed to Tolclofos-methyl

Tolclofos-methyl concentration [µg a.s./L]		Cumulative mean number of dead animals (%)[1)]					Observations[2)]
Nominal	Mean Measured	4.5 h	24h	48h	72h	96h	
Negative control	---[3)]	0	0	10	10	10	AN
Solvent control	---[3)]	0	0	0	0	0	AN
63	60	0	5	5	5	5	AN
125	121	0	0	0	0	0	AN
250	230	0	0	5	15	15	AN
500	458	0	10	50	50	65	AN/S
1000	742	0	35	95	100	100	n.d.

[1)] Dead and missing animals (missing animals assumed dead). Mean from 2 replicates and cumulative values presented

[2)] Observations at end of the test; AN = appeared normal, S = smaller in comparison to control

[3)] smaller LOQ (30.0 µg a.s./L) n.d. not determined, due to 100% mortality

EC_{50} values and NOEC re-calculated by the applicant (Wirzinger and Strecker, 2017) based on geomean measured concentrations are presented in the table below.

Table 81: Toxicity endpoints for effects on Americamysis bahia (Palmer et al., 2010) after exposure to Tolclofos-methyl

Parameter	**EC_{50} [µg a.s./L]**	**NOEC [µg a.s./L]**
48 h mortality (95 % confidence intervals)	444 (381 – 517)	229
96 h mortality (95 % confidence intervals)	378 (324 – 442)	229

Conclusion

The mean measured value of the highest concentration (1000 µg/L) was only 74% of the nominal concentration. However, the results were based on the measured concentrations and are therefore considered acceptable.

The study fulfils the validity criteria of OPPTS 850.1035 with a mortality ≤10% in the control. The RMS agrees with the conclusions made by the applicant. The 96 hours EC_{50} value is 0.377 mg a.s./L.

It can be summarized that tolclofos-methyl is very toxic to invertebrates with LC_{50} < 1 mg/L with the most sensitive species the saltwater mysid *Americamysis bahia* with 96h EC_{50} =0.377 mg/L.

Metabolites

The tolclofos-methyl metabolite pH-CH_3, was tested with the species salt water mysids (*Americamysis bahia*) and the EC_{50} was calculated after 96 hours. This value will be used for classification purpose.

The other study on the tolclofos-methyl metabolite DM-TM the EC_{50} could not be used for classification purpose since the value was expressed as EC_{50}> 100 mg metabolite/L.

Reference:	Shaw, A.C. (2014b) ph-CH_3 – Acute Toxicity to Mysid (*Americamysis bahia*) Under Static Conditions, Following OCSPP Draft Guideline 850.1035
Company Report No.:	QW-0148
	13048.6803 (825-006)
Guideline:	OPPTS 850.1035
	U.S EPA Guideline
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal
Material and methods:	
Test material:	ph-CH_3
Lot/Batch No:	ZDNWA
Purity:	97.7 %
Species:	Saltwater mysids (*Americamysis bahia*)
Test media:	Natural filtered seawater (19-20‰).
Treatments:	Nominal: 0 (control), 0.13, 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg a.i./L
Duration:	96 h
Exposure:	Each test vessel (glass beaker; 1 L) was filled with 0.9 L of test water. Two replicate vessels for each treatment and control with 10 animals in each replicate, resulting in a total of 20 animals per treatment and control. The animals were <24h old at the start of the test.
Test conditions:	Photoperiod 16:8 light:dark
	Temperature: 24-25°C
	pH: 7.7-7.9
	Dissolved oxygen: 5.3-7.4 mg/L
Observations:	Mortality and abnormal behaviour were evaluated after 0, 24, 48, 72 and 96 hours.
Chemical analysis:	Chemical analyses of ph-CH_3 were performed using a high performance liquid chromatography with ultraviolet detection (HPLC/UV). Average recovery rate in filtered seawater was 107 ± 2.25% with a LOQ of 0.05 mg/L. Samples were taken at exposure initiation and exposure termination.
Data analysis:	EC_{50} values were calculated using CETIS™ Version 1.8.

Results:

The results of the chemical analysis for ph-CH_3 are presented in Table 8.2.4.2-8. The arithmetic mean measured concentrations were determined to be were 0.12, 0.24, 0.64, 0.96, 2.0, 4.0 and 8.0 mg ph-CH_3/L, representing 95, 95, 130, 96, 99, 100 and 100 % of the nominal concentrations, respectively.

Table 82: Measured ph-CH3 concentrations in the exposure solutions during the 96 hour test with *Americamysis bahia*

Nominal concentration [mg/L]	Measured concentration [mg/L] [a]			
	0 hours (new)	96 hours (spent)	Arithm. mean concentration	Geomean concentration
0 (control)	< LOQ	< LOQ	-	-
0.13	0.13	0.11	0.12 (95)	0.12 (92.0)
0.25	0.26	0.21	0.24 (95)	0.23 (93.5)
0.50	0.70	0.58	0.64 (130)	0.64 (127)
1.0	1.0	0.89	0.96 (96)	0.94 (94.3)
2.0	2.1	1.8	2.0 (99)	1.9 (97.2)
4.0	4.2	3.8	4.0 (100)	4.0 (99.9)
8.0	8.6	7.5	8.0 (100)	8.0 (100)

[a] values in parentheses represent % of nominal concentration

LOQ = 0.0063 mg/L

Conclusion

No mortality was observed in the control or the two lowest concentrations. In the 0.64, 0.96 and 2.0 mg ph-CH_3/L treatment levels the observed mortalities after 96 hours were 20, 40 and 85 %, respectively. At test end, after 96 hours, the mortality in the two highest treatment levels was 100 %.

Table 83: Summary of mortality of saltwater mysids (*Americamysis bahia*) exposed to ph-CH_3

Nominal concentration [mg/L]	Mean measured concentration [mg/L]	Cumulative mean number of dead animals (%)[1)]			
		24h	48h	72h	96h
Control	NA	0	0	0	0
0.13	0.12	0	0	0	0
0.25	0.24	0	0	0	0
0.50	0.64	0	10	20	20
1.0	0.96	15	35	40	40
2.0	2.0	65	85	85	85
4.0	4.0	100	100	100	100
8.0	8.0	100	100	100	100

[1)] Dead animals; mean from 2 replicates and cumulative values presented

The calculated EC_{50} values and NOEC were re-calculated by the applicant based on the geomean measured test concentrations (Wirzinger *et al*., 2017b) and the results are presented in the table below.

Table 84: Toxicity endpoints for effects on Americamysis bahia (Shaw, 2014) after exposure to the Tolclofos-methyl metabolite ph-CH_3

Parameter	EC_{50} [mg/L]	NOEC [mg/L]
48 h mortality (95 % confidence intervals)	1.2 (0.96 – 1.4)	0.23
96 h mortality (95 % confidence intervals)	1.1 (0.87 – 1.3)	0.23

It can be concluded that the 96-hour EC50 for the saltwater mysid *Americamysis bahia* was 1.1 mg/L of ph-CH_3. This study is accepted and fulfils the validity criteria of OPPTS 850.1035 as the mortality in control was <10%.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Reference:	Sayers, L.E. (2003) Tolclofos-methyl – Toxicity to the Freshwater Green Alga, *Scenedesmus subspicatus* Wirzinger, G, Schmitz, A. (2014) Re-calculation of Toxicity Endpoints for Effects of Tolclofos-methyl on Algae
Company Report No.:	QW-0072 13048.6415 (994-08025) and QW-0144 PP107-20010 (882-001)
Guideline:	OECD No. 201, EC Guideline Annex V - Method C.3. OECD 201 (2011) (for the re-calculations)
GLP:	Yes (not for the re-calculations)
Previous evaluation:	Laboratory part included in DAR (2003) Re-calculation is submitted for the purpose of renewal

Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	00668G
Purity:	97.8 %
Species:	*Scenedesmus subspicatus*
Test system/test conditions:	Tested concentrations were control, solvent control (0.1 mL/L acetone), 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L. Algal Assay Procedure (AAP) medium was used as test media and all controls and treatments were tested in replicates of three with an initial (0 hour) cell density of 1.0 x 10^4 cells/mL. Cell counts (cells/ml) were made at 24-hour intervals using a

	haemocytometer and observations on the health of the algal cells were also made at each 24-hour interval. The test was terminated after 72 hour. The temperature ranged between 23-24°C and the pH was 7.3 at the start and 8.6-9.7 at the end of the test. The test was conducted at a light intensity of 7000-8900 lux and shaking at 100 rpm. Samples for chemical analyses were taken at test initiation and a test termination and analysed using HPLC/UV (LOQ = 0.050 mg a.s./L). An additional sample from the highest concentration was removed and centrifuged prior to analysis.
Data analysis:	The data of the cell densities and the mean measured concentrations provided in the report of Sayers (2003) were used to re-calculate the validity criteria and the respective endpoints (E_rCx and E_yCx values, NOEC, LOEC) by using the program ToxRat® professional (2.10), ToxRat® solutions.

Results:

The mean measured concentrations were 0.13, 0.22, 0.39, 0.69 and 1.1 mg a.s./L (97-53% of the nominal concentrations). In the highest concentration (2.0 mg a.s./L), a small amount of undissolved test substance was observed. After centrifugation, the measured concentration in this test solution was 0.7 mg a.s./L at 0 h, which corresponds to the water solubility of tolclofos-methyl. The presence of undissolved substance was not considered to have an effect on the growth rate.

Table 85: Analytical results of tolclofos-methyl concentrations in the test medium.

Nominal concentration (mg a.i./L)	Measured concentration (mg a.i./L)			
	0 hour	72 hour	Geomean	Percent of nominal
Control	<0.014	<0.015	NA	NA
Solvent control	<0.014	<0.015	NA	NA
0.13	0.16	0.094	0.12	94.3
0.25	0.26	0.18	0.22	86.5
0.50	0.42	0.35/0.32[c]	0.38	76.7
1.0	0.89	0.49	0.66	66.0
2.0	1.6/0.70[c]	0.51	0.90	45.2

[C] Centrifuged samples.

The reported test conditions (dissolved oxygen concentration and temperature) in the definitive test were within expected ranges. Only the reported shift of the pH value in the control was more than 1.5 units (actual shift: 2.2) as recommended by the guideline. However, it was proposed by the study authors not to have any influence on the test results as the algae in the control showed good performance. There is no information on a pH dependent hydrolysis of tolclofos-methyl.

As there were no statistical differences between control and solvent control, the results for the different parameters were evaluated by comparison against the solvent control.

Table 86: Yield of Scenedesmus subcapitatus after exposure to tolclofos-methyl

Mean measured concentration [mg a.s./L]	Yield [x 10^4 cells/mL] (percent inhibition)		
	24 h	48 h	72 h
Solvent control	4.00	15.25	66.92
0.13	3.75 (6.3)	21.08 (-38.3)	55.25 * (17.4)
0.22	3.17 (20.8)	18.25 (-19.7)	51.67 * (22.8)
0.39	3.00 (25.0)	12.67 (16.9)	39.75 * (40.6)
0.69	2.17 * (45.8)	10.75 * (29.5)	29.33 * (56.2)
1.1[a]	1.00 * (75.0)	7.83 * (48.6)	17.08 * (74.5)

Values in parentheses represent percent inhibition compared to solvent control

Negative values indicate a higher yield in treatment group compared to solvent control

* Statistically significantly different compared to control ($p \leq 0.05$, Williams t-test)

[a] Non-centrifuged sample

Table 87: Growth rate of *Scenedesmus subcapitatus* after exposure to tolclofos-methyl

Mean measured concentration [mg a.s./L]	Growth rate [x 10^4 cells/mL/d] (percent inhibition)		
	24 h	48 h	72 h
Solvent control	1.57	1.39	1.40
0.13	1.56 (0.8)	1.55 (-11.4)	1.34 (4.2)
0.22	1.42 (9.3)	1.47 (-6.0)	1.32 * (5.9)
0.39	1.36 (13.5)	1.30 (6.5)	1.23 * (12.0)
0.69	1.13 * (28.3)	1.23 (11.6)	1.14 * (19.0)
1.1[a]	0.672 * (57.2)	1.07 * (23.2)	0.961 * (31.4)

Values in parentheses represent percent inhibition compared to solvent control

Negative values indicate a higher yield in treatment group compared to solvent control

* Statistically significantly different compared to control ($p \leq 0.05$, Williams t-test)

[a] Non-centrifuged sample

The reported effect concentrations are presented in the table below.

Table 88: Effect concentrations reported in the original study (Sayers, 2003) and the re-calculated effect concentrations based on geomean measured values.

	Endpoints as presented in original report (Sayers, 2003) [mg a.i./L]	**Re-calculated endpoints [mg a.i./L]**
Yield		
EC_{10}	n.r.	0.10
EC_{20}	n.r.	0.18
EC_{50}	n.r.	0.49

Mean measured concentration	Growth rate [x 10^4 cells/mL/d] (percent inhibition)	
NOEC	n.r.	0.12
Growth rate		
EC_{10}	n.r.	0.34
EC_{20}	n.r.	0.67
EC_{50}	> 1.1 (estimated)	0.90*
NOEC	0.22	0.12

n.r. = not reported

* could not be determined due to mathematical reasons and was therefore estimated to be greater than the highest test concentration

Conclusion

The laboratory study with toxicity-testing of *Scenedesmus subspicatus* was accepted without any further comments in DAR (2003) and both parts of this study are well performed and reported. However, since there are new validity criteria for toxicity testing with algae (OECD TG 201, 2011), the applicant has conducted new calculations based on the existing data.

Even though the initial biomass concentration is higher than recommended in the standard (1.0 x 10^4 cells/mL, compared to the recommended 2-5 x 10^3 cells/mL), the validity criteria were fulfilled according to OECD TG 201 (2011) with an average specific growth rate >0.92 day^{-1}. Furthermore, the coefficient of variation of average growth rate was <7% for 0-72 h and <35% for the section-by-section specific growth rate.

Table 89: 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 (%)
Control	1					1.33	0.15	0.12		
	2	1.34	0.017	0.012	1.25	1.30	0.26	0.20	0.219	21.9
	3					1.33	0.45	0.34		
Solvent control	1					1.40	0.44	0.31		
	2	1.40	0.068	0.048	4.84	1.33	0.12	0.087	0.210	21.0
	3					1.50	0.35	0.23		

According to OECD TG 23 (2000), reported effect concentrations cannot be higher than saturation concentration. Based on the measured concentration at 0 h in the centrifuged sample, the correct E_rC_{50} for growth rate should therefore be >0.7 mg a.i./L. The value of this growth rate dcn however not be used for classification purpose. Instead the EC_{50} of biomass will be used.

Overall it can be concluded that tolclofos-methyl is very toxic to the algae *Scenedesmus subspicatus* with a 72 h EC_{50} (biomass)=0.49 mg/L and NOEC =0.12 mg a.s/L

Reference:	**Hoberg, J.R. (1998)** Desmethyl-Tolclofosmethyl – Toxicity to the freshwater green alga, *Scenedesmus subspicatus* **Wirzinger & Ruhnke (2016)** Re-calculation of toxicity endpoints for effects of the metabolite DM-TM on algae
Company Report No.:	QW-0053; QW-0157 13048.1198.6176.460 (994-08026)
Guideline:	OECD No. 201
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	DM-TM
Lot/Batch No:	CTS98009G
Purity:	98.9 %
Species:	*Scenedesmus subspicatus*
Test system/test conditions:	Tested concentrations were control, 8.0, 16, 31, 63 and 125 mg/L. Algal Assay Procedure (AAP) medium was used as test media and all controls and treatments were tested in replicates of three with an initial (0 hour) cell density of 1.0 x 10^4 cells/mL. Cell counts (cells/ml) were made at 24-hour intervals using a haemocytometer and observations on the health of the algal cells were also made at each 24-hour interval. The test was terminated after 72 hour. The temperature ranged between 24-25°C and the pH was 7.4 at the start and 9.6-9.7 at the end of the test. The test was conducted at a light intensity of 4100-5000 lux and shaking at 100 rpm. Samples for chemical analyses were taken at test initiation and a test termination and analysed using HPLC/UV (LOQ = 0.108 mg a.s./L). Effects on growth rate and biomass were analysed using Williams test and linear regression.

Results:

Measured concentrations (6.0, 12, 23, 34, 48 and 97 mg/L) corresponded to 75-77% of the nominal.

The results of the chemical analysis for DM-TM are presented in the table below. The mean measured concentrations were determined to be 6.0, 12, 23, 48, and 97 mg a.s./L, representing 75, 77, 75, 76 and 77 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 90. Measured DM-TM concentrations in the exposure solutions during the 72-hour exposure of Desmodesmus subspicatus

Nominal concentration [mg/L]	Measured concentration [mg/L] a			
	0 hour (new)	72 hour (spent)	Arithm. mean concentration	Geomean concentration
0 (control)	< 0.55	< 0.52	-	-
8.0	6.0	6.0	6.0 (75)	6.0 (75)
16	12	12	12 (77)	12 (77)
31	24	23 / 24b	23 (75)	24 (76)
63	49	48	48 (76)	49 (77)
125	98	95	97 (77)	97 (77)

a values in parentheses represent % of nominal concentration

b result of additional sample without algae present

For the 72-hour biomass results, test concentrations of 23 mg/L and above were significantly reduced as compared with the control. The 0-72 hour growth rates at test concentrations of 48 mg/L and above were significantly reduced compared with the control.

Table 91. Cell density and growth parameters of the algal growth inhibition test of DM-TM with Scenedesmus subspicatus

Mean measured concentration (mg/l)	Cell density (x10^4 cells/ml)[1]			72-hour biomass (x10^4 cells days/ml)		0-72 hour growth rate (days^{-1})	
	24-hour	48-hour	72-hour	Total area[1]	[% inhibition]	Growth rate[1]	[% inhibition]
Control	9.5(0.25)	98(1.0)	139(3.4)	165(2.2)	-	1.67(0.008)	-
6.0	9.6(0.63)	99(2.2)	142(1.4)	167(3.2)	-1.0	1.68(0.003)	-0.48
12	7.6(0.38)	98(1.3)	138(2.4)	162(1.6)	1.9	1.67(0.006)	0.12
23	6.5(0.43)	95(2.1)	136(1.6)	158(2.9)[2]	4.6	1.66(0.004)	0.42
48	7.1(1.0)	94(1.5)	133(0.72)	156(1.8)[2]	5.9	1.65(0.003)[2]	0.90
97	6.5(1.4)	91(0.8)	131(1.2)	151(1.0)[2]	8.6	1.65(0.003)[2]	1.3

[1]: Mean (SD)

[2]: Significantly reduced compared to the control (Williams' Test, P≤0.05)

Based on the results of this study the acute toxicity (72-hour EC_{50}) of DM-TM to the alga *Scenedesmus subspicatus* could not be obtained and was therefore concluded to be >97 mg/L. The no-observed effect concentration (NOEC) for biomass and growth rate was reported as 12 and 23 mg/L, respectively.

RMS comments and conclusion:

In DAR (2003) the study was accepted without remarks. In the re-assessment of tolclofos-methyl it was shown that this study does not fulfil the updated validity criteria of OECD TG 201 (2011), which was also acknowledged by the applicant. The biomass should increase exponentially during 72 hours and increase by a factor of 16, corresponding to a specific growth rate of 0.92 day^{-1}. Even though the biomass increased with a factor of 136, the growth was not exponential (see graph) due to low growth rate during the last 24 hour. This decreased growth rate also resulted in a high coefficient of variation for the section-by-section of 68% (it should not be higher than 35%).

Table 92. 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 (%)
Control	1	1.64	0.0072	0.0044	0.44	1.64	1.13	0.69	0.68	68%
	2					1.65	1.12	0.68		
	3					1.65	1.13	0.69		

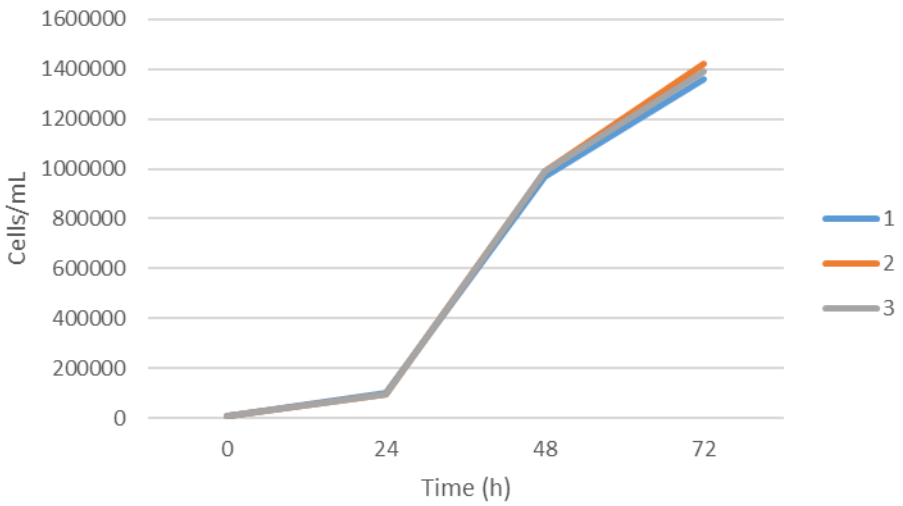


Figure B.9.2.6-1. Growth in individual replicates in the control cultures.

However, according to the OECD TG 201 (2011), the test duration could be shortened to 48 hours if an increase in cell density by a factor of 16 was obtained. If the cell density of 48 hours were used to assess toxicity instead, the E_rC_{50} would still be >97 mg/L. The validity criteria of OECD TG 201 (2004) were fulfilled for the 48 h data and the study is therefore considered acceptable with the shortened exposure time.

Table 93. Average coefficient of variance at 0-48 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 (%)
Control	1 2 3	2.29	0.0059	0.0026	0.26	2.29 2.30 2.30	0.007 0.096 0.065	0.003 0.042 0.028	0.022	2.2%

11.5.4 Acute (short-term) toxicity to other aquatic organisms

All the information on acute toxicity are taken from the RAR and list of endpoints for tolclofos-metyl, October 2017.

11.6 Long-term aquatic hazard

Table 94: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4	*Oncorhynchus mykiss* Rainbow Trout	Tolclofos-methyl	Chronic (flow-through, 97 days) Growth, NOEC =0.012 mg a.s./L EC_{10} =0.013 mg a.s./L	Reliability 1	Xxxx (1991) QW-11-0040; Wirzinger ans Ruhnke (2016) QW-0163

U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4	*Daphnia magna* Water flea	Tolclofos-methyl	21 d(flowthrough) Reproduction NOEC=0.026 mg a.s./L EC_{10}=0.036 mg a.s./L (mm)	Reliability 1	Burgess, D (1989) QW-91-0031; Wirzinger and Ruhnke (2016) QW-0159
OECD Draft TG 219 (2001)	*Chironomus riparius* Midget larvea	Tolclofos-methyl	28 d (static water/sediment system) Development NOEC=0.25 mg/l EC_{10}=0.62 mg a.s./L	Reliability 1	Putt, A.E. (2002) QW-0063; Wirzinger and Ruhnke (2016), QW-0162
OECD No. 201, EC Guideline Annex V - Method C.3. OECD 201 (2011) (for the re-calculations)	*Scenedesmus subspicatus*	Tolclofos-methyl	72 h (static) EyC_{50}=0.49 mg a.s. /L EC_{50} (growth) no information) NOEC =0.12 mg a.s/L	Reliability 1	Sayers, L.E. (2003) QW-0072 and Wirzinger,G *et al.* (2014) QW-0144
OECD No. 201, EC Guideline Annex V - Method C.3.	*Scenedesmus subspicatus*	DM-TM	Chronic 48 h (static)	Reliability 1	Hoberg, J.R. (1998) QW-0053; Wirzinger & Ruhnke (2016), QW-0157

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

Reference:	xxxx (1991) Early Life Stage Toxicity of Rizolex® Technical to Rainbow Trout (*Oncorhynchus mykiss*) in a Flow-Through System Wirzinger, G, Ruhnke, H (2016) Re-calculation of toxicity endpoints from a fish early life stage test on effects of Tolclofos-methyl on rainbow trout
Company Report No.:	QW-11-0040 38586R (994-08020) QW-0163
Guideline:	U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl

Lot/Batch No:	40810
Purity:	97.6%
Species:	*Oncorhynchus mykiss*
Test system/test conditions:	A flow-through system (flow rate approx. 92 L/day/replicate) with glass chambers of 11.7 L was used. Tested concentrations were control, solvent control, 0.013, 0.025, 0.050, 0.100 and 0.200 mg/L. Four replicates with 35 eggs each per concentration, control and solvent control. 200 eggs were incubated separately and checked for viability at day 11. Hatching was observed daily after day 32. Reduction to 15 fry per replicates was made on day 40. Behavioural and physical changes as well as mortality were monitored daily. At termination (day 97, 60 days post-hatch), standard length and wet weight of the fry were measured. The temperature was 8.6-11.2°C, pH 8.0-8.7 and dissolved oxygen concentration 8.1-10.8 mg/L. Egg hatchability and survival were analysed using Fisher´s exact test. Growth data was analysed by ANOVA. Samples for chemical analyses were taken weekly and analyzed using gas-liquid chromatography with an electron capture detector (LOQ not available but according to the applicant, LOQ was stated to be 0.0025 mg/L). ECx values were calculated with regression analysis.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean measured concentrations were determined to be 0.012, 0.028, 0.053, 0.110, and 0.200 mg a.s./L, representing 92, 112, 106, 110 and 100 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 95: Measured Tolclofos-methyl concentrations in the exposure solutions during the fish early life cycle test of *Oncorhyncus mykiss*

	Nominal concentration [mg a.s./L]						
	Control	Solvent Control	0.013	0.025	0.050	0.100	0.200
Sampling Day	Measured concentration [mg a.s./L]						
0	< LOQ	< LOQ	0.011	0.024	0.044	0.091	0.150
1	n.d.	n.d.	0.014	0.027	0.053	0.101	0.190
7	n.d.	n.d.	0.011	0.024	0.049	0.096	0.190
14	n.d.	n.d.	0.011	0.025	0.050	0.094	0.190
21	n.d.	n.d.	0.012	0.030	0.059	0.120	0.230
28	< LOQ	< LOQ	0.011	0.025	0.051	0.098	0.200
35	n.d.	n.d.	0.011	0.027	0.053	0.110	0.190
42	n.d.	n.d.	0.014	0.034	0.059	0.130	0.230
49	n.d.	n.d.	0.013	0.030	0.053	0.110	0.210
56	n.d.	n.d.	0.013	0.032	0.057	0.120	0.220
63	n.d.	n.d.	0.012	0.025	0.051	0.093	0.200
70	n.d.	n.d.	0.013	0.028	0.055	0.110	0.220
77	n.d.	n.d.	0.012	0.027	0.052	0.095	0.200
84	n.d.	n.d.	0.012	0.030	0.061	0.120	0.240
91	n.d.	n.d.	0.011	0.027	0.052	0.097	0.200
97	n.d.	n.d.	0.012	0.027	0.054	0.110	0.210
Mean [a]			0.012 (92)	0.028 (112)	0.053 (106)	0.110 (110)	0.200 (100)

[a] values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 0.0025 mg a.s./L

Swim-up progressed somewhat slower at test concentrations of 0.028 and 0.053 mg/L, but only at concentrations of 0.110 and 0.200 mg/L did there appear to be a biologically significant delay in swim-up. The incidence of sublethal abnormalities (e.g. dark discoloration, swimming vertically in chamber and surfacing) was much greater in the two highest test concentrations and was considered to be dose-related.

Table 96: Egg hatchability, fry survival, standard length and wet weight of rainbow trout (*Oncorhynchus mykiss*) exposed to tolclofos-methyl during the early life stage toxicity test

Nominal Concentration (mg/l)	Mean Measured Concentration (mg/l)	Egg Hatchability (%)	60-Day Post-Hatch		
			Survival (%)	Standard length (mm)	Wet weight (g)
Control	0	100	92	46.6	1.677
Solvent control	0	100	100	45.1	1.541
0.013	0.012	99	98	44.0	1.440
0.025	0.028	99	95	43.2	1.359[*2]
0.050	0.053	99	100	39.0[*2]	1.036[*2]
0.100	0.110	99	85[*1]	31.3[a]	0.511[a]
0.200	0.200	100	17[*1]	25.2[a]	0.274[a]

[a]: Data not included in growth analysis since a survival effect had been determined at this level.

[*1]: Statistically significant reduction compared to solvent control (Fisher's exact test, P<0.05).

[*2]: Statistically significant reduction compared to solvent control (Nested ANOVA and Dunnett's test, P<0.05).

Based on the observed effects on growth, the 97-day no-observed effect concentration (NOEC) for technical tolclofos-methyl to the early life-stages of rainbow trout was 0.012 mg/L (mean measured).

Table 97: Effect values with confidence intervals for rainbow trout (*Oncorhynchus mykiss*) exposed to tolclofos-methyl during the early life stage toxicity test

Parameter	EC_{10} [mg a.s./L]	EC_{20} [mg a.s./L]	EC_{50} [mg a.s./L]
Hatchability (95 % confidence intervals)	n.d.	n.d.	n.d.
Post-hatch survival (60 d) (95 % confidence intervals)	0.104 (0.059 – 0.140)	0.124 (0.017 – 0.160)	0.162 (0.078 – 0.204)
Fresh weight (95 % confidence intervals)	0.013 (0.0066 – 0.017)	0.026 (0.020 – 0.032)	0.109 (0.078 – 0.214)
Length (95 % confidence intervals)	0.037 (0.033 – 0.041)	0.075 (0.065 – 0.095)	> 0.200 *

* the calculated value was higher than the highest concentration tested, thus the EC_{50} is reported as unbound value

n.d. not determined due to mathematical reasons

Conclusion

A temporary deviation from the accepted temperature occurred, however, this was corrected as soon as possible. The validity criteria of OECD TG 210 (2013) are otherwise fulfilled.

The EC_x values had large confidence intervals, thus there is an uncertainty regarding these values. However, this will not affect the classification since the lowest effect value will be used (NOEC = 0.012 mg/L).

In conclusion tolclofos-methyl has a long-term toxicity towards fish with the most sensitive species *Oncorhynchus mykiss* (Rainbow Trout) NOEC (97 days)=0.012 mg/l.

11.6.2 Chronic toxicity to aquatic invertebrates

All the information on chronic toxicity are taken from the RAR and list of endpoints for tolclofos-metyl, October 2017.

11.6.2.1 Long-term and chronic toxicity to aquatic invertebrates

Reference:	Burgess, D. (1989) Chronic Toxicity of Rizolex to *Daphnia magna* Under Flow-Through Test Conditions Wirzinger, G, Ruhnke, H (2016) Re-calculation of toxicity endpoints for effects of Tolclofos-methyl on *Daphnia magna*
Company Report No.:	QW-91-0031 37552 (994-08017) QW-0159
Guideline:	U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	80410
Purity:	97.6%
Species:	*Daphnia magna*
Test system/test conditions:	The 21 days test was performed by use of a flow-through system at a 16:8 h light:dark photoperiod. Tested concentrations were control, solvent control, 0.024, 0.048, 0.10, 0.20 and 0.40 mg/L. Test solutions were analysed at 0, 4, 7, 10, 14, 17 and 21 days. Temperature was 20-21°C, pH 7.4-8.1, dissolved oxygen 5.4-8.2 mg/L. 10 first instar daphnids were placed individually in scintillation vials and transferred to the replicate test chambers. In total 40 daphnids were exposed to each control and test level. The daphnids were fed with *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) three times per day. Observations were made regarding survival, abnormal effects, time to first brood, number of offspring produced during the study and length of adult daphnids at day 21. ECx values were calculated with regression analysis.

Results:

The results of the chemical analysis for Tolclofos-methyl are presented in the table below. The mean measured concentrations were determined to be 0.026, 0.062, 0.089, 0.19, and 0.39 mg a.s./L, representing 108, 129, 89, 95, and 98 % of the nominal concentrations, respectively. The results of the study were based on the arithmetic mean measured concentrations.

Table 98: Measured Tolclofos-methyl concentrations in the exposure solutions during the 21-day chronic life cycle test with *Daphnia magna*

Sampling Day	Nominal concentration [mg a.s./L]						
	Control	Solvent control	0.024	0.048	0.10	0.20	0.40
	Measured concentration [mg a.s./L]						
0	< LOQ	< LOQ	0.024	0.056	0.078	0.19	0.36
4	< LOQ	< LOQ	0.025	0.060	0.083	0.17	0.39
7	< LOQ	< LOQ	0.033	0.075	0.11	0.24	0.41
10	< LOQ	< LOQ	0.024	0.057	0.079	0.15	0.38
14	< LOQ	< LOQ	0.029	0.069	0.10	0.21	0.39
17	< LOQ	< LOQ	0.026	0.058	0.091	0.17	0.41
21	< LOQ	< LOQ	0.025	0.063	0.089	0.19	0.39
Mean [a]	-	-	0.026 (108)	0.062 (129)	0.089 (89)	0.19 (95)	0.39 (98)

[a] values in parentheses represent % of nominal concentration

n.d. not determined

LOQ = 0.0112 mg a.s./L

Table 99: Survival, number of young/adult reproduction day, time to first brood and adult length of *Daphnia magna* exposed to tolclofos-methyl during a 21 days life cycle test

Mean Measured Concentration (mg/l)	Mean Survival (%)	Adult Mean Length (mm)	Time to First Brood (days)	Mean Young/Adult Reproduction Day
Control	93	3.5	9.0	3.8
Solvent control	95	3.6	8.5	4.7
0.026	98	3.5	8.8	4.2
0.062	95	3.3*	8.5	3.2*
0.089	80	3.2*	9.0	3.1*
0.19	35*	2.7*	9.0	0.8*
0.39	0*	-	-	-

* Values significantly different (P<0.05) from the solvent control using one-way analysis of variance (ANOVA) and Dunnett's t-test or Tukey's HSD Multiple Means Comparison Test.

Effect values, based on the arithmetic mean measured concentrations, are presented in the table below. NOEC for reproduction was set to 0.026 mg a.i./L.

Table 100: Effect values for *Daphnia magna* exposed to tolclofos-methyl during a 21 days life cycle test

Parameter	EC_{10} [mg/L]	EC_{20} [mg/L]	EC_{50} [mg/L]
Mortality (95 % CI)	0.090 (0.073 – 0.104)	0.108 (0.091 – 0.123)	0.155 (0.137 – 0.177)
Reproduction (offspring per introduced parent) (95 % CI)	0.036 (0.030 – 0.041)	0.049 (0.043 – 0.054)	0.088 (0.082 – 0.095)
Reproduction (offspring per survived parent) (95 % CI)	0.034 (0.029 – 0.039)	0.050 (0.044 – 0.055)	0.101 (0.095 – 0.108)
Growth (length) (95 % CI)	0.088 (0.070 – 0.161)	0.178 (0.118 – 2.141)	0.689 (0.264 – n.d.)

Conclusion

It is not possible to extract information regarding total number of offspring/adult for each individual and thus judge whether this study fulfills the validity criteria of OECD TG 211 (2012). The RMS made an estimated calculation of the mean number of offspring/adult for the control and solvent control according to the table below.

Table 101: Calculated total number of offspring/adult for the control and solvent control.

	Time to first brood (day)	Mean offspring/adult Reproductive day	Reproductive days[a]	Total number of offspring/adult
Control	9	3.8	12	45.6
Solvent control	8.5	4.7	12.5	58.75

[a]Time of the exposure (21 days) subtracted with time to first brood

According to this calculation, the number of offspring/adult was <60 and thus does not fulfil the validity criteria of OECD TG 211 (2012). However, the total number of offspring in the solvent control is close to the validity criteria of ≥60 living offspring per surviving parent and the study is therefore accepted.

The validity of the study summarised above (Burgess, 1989) was questioned during the peer review, and therefore the applicant provided the following additional information:

"For the number of offspring the control and the solvent control were statistical significantly different. Thus according to OECD 211, the solvent control must be used for the evaluation. The mean number of offspring per adult in the solvent control was 64 (please refer to Wirzinger & Ruhnke (2016), QW-0159), and therefore the study is valid.

According to OECD guideline 211, generally in a well-run test, the coefficient of variation (CV) around the mean number of living offspring produced per parent animal in the control(s) should be ≤ 25%. In the chronic Daphnia magna study with Tolclofos-methyl (Burgess, 1989), CV around the mean number of living offspring produced per parent animal in solvent control are 1.4%. Additionally, the mortality in the control was 7.5 %. Therefore, the daphnid population used in this study was considered to be sufficiently healthy and the test was considered to be reliable." The RMS agree with the applicant. The validity of the study was re-evaluated against OECD 211 by Wirzinger and Ruhnke (2016) and according to this evaluation the criteria was fulfilled.

In conclusion tolclofos-methyl is very toxic towards invertebrates with the most sensitive *Daphnia magna* 21 day NOEC=0.026 mg/L.

11.6.3 Chronic toxicity to algae or other aquatic plants

Since there was no chronic algae study, the NOEC from the acute aquatic alge study was used.

Reference:	Sayers, L.E. (2003) Tolclofos-methyl – Toxicity to the Freshwater Green Alga, *Scenedesmus subspicatus* Wirzinger, G, Schmitz, A. (2014) Re-calculation of Toxicity Endpoints for Effects of Tolclofos-methyl on Algae
Company Report No.:	QW-0072 13048.6415 (994-08025) and QW-0144 PP107-20010 (882-001)
Guideline:	OECD No. 201, EC Guideline Annex V - Method C.3. OECD 201 (2011) (for the re-calculations)
GLP:	Yes (not for the re-calculations)
Previous evaluation:	Laboratory part included in DAR (2003) Re-calculation is submitted for the purpose of renewal
Material and methods:	
Test material:	Tolclofos-methyl
Lot/Batch No:	00668G
Purity:	97.8 %
Species:	*Scenedesmus subspicatus*
Test system/test conditions:	Tested concentrations were control, solvent control (0.1 mL/L acetone), 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L. Algal Assay Procedure (AAP) medium was used as test media and all controls and treatments were tested in replicates of three with an initial (0 hour) cell density of 1.0 x 10^4 cells/mL. Cell counts (cells/ml) were made at 24-hour intervals using a haemocytometer and observations on the health of the algal cells were also made at each 24-hour interval. The test was terminated after 72 hour. The temperature ranged between 23-24°C and the pH was 7.3 at the start and 8.6-9.7 at the end of the test. The test was conducted at a light intensity of 7000-8900 lux and shaking at 100 rpm. Samples for chemical analyses were taken at test initiation and a test termination and analysed using HPLC/UV (LOQ = 0.050 mg a.s./L). An additional sample from the highest concentration was removed and centrifuged prior to analysis.

Data analysis:	The data of the cell densities and the mean measured concentrations provided in the report of Sayers (2003) were used to re-calculate the validity criteria and the respective endpoints (E_rC_x and E_yC_x values, NOEC, LOEC) by using the program ToxRat® professional (2.10), ToxRat® solutions.

Results:

The mean measured concentrations were 0.13, 0.22, 0.39, 0.69 and 1.1 mg a.s./L (97-53% of the nominal concentrations). In the highest concentration (2.0 mg a.s./L), a small amount of undissolved test substance was observed. After centrifugation, the measured concentration in this test solution was 0.7 mg a.s./L at 0 h, which corresponds to the water solubility of tolclofos-methyl. The presence of undissolved substance was not considered to have an effect on the growth rate.

Table 102: Analytical results of tolclofos-methyl concentrations in the test medium.

Nominal concentration (mg a.i./L)	Measured concentration (mg a.i./L)			
	0 hour	72 hour	Geomean	Percent of nominal
Control	<0.014	<0.015	NA	NA
Solvent control	<0.014	<0.015	NA	NA
0.13	0.16	0.094	0.12	94.3
0.25	0.26	0.18	0.22	86.5
0.50	0.42	0.35/0.32[c]	0.38	76.7
1.0	0.89	0.49	0.66	66.0
2.0	1.6/0.70[c]	0.51	0.90	45.2

[C] Centrifuged samples.

The reported test conditions (dissolved oxygen concentration and temperature) in the definitive test were within expected ranges. Only the reported shift of the pH value in the control was more than 1.5 units (actual shift: 2.2) as recommended by the guideline. However, it was proposed by the study authors not to have any influence on the test results as the algae in the control showed good performance. There is no information on a pH dependent hydrolysis of tolclofos-methyl.

As there were no statistical differences between control and solvent control, the results for the different parameters were evaluated by comparison against the solvent control.

Table 103: Yield of *Scenedesmus subcapitatus* after exposure to tolclofos-methyl

Mean measured concentration [mg a.s./L]	Yield [x 10^4 cells/mL] (percent inhibition)		
	24 h	48 h	72 h
Solvent control	4.00	15.25	66.92
0.13	3.75 (6.3)	21.08 (-38.3)	55.25 * (17.4)
0.22	3.17 (20.8)	18.25 (-19.7)	51.67 * (22.8)
0.39	3.00 (25.0)	12.67 (16.9)	39.75 * (40.6)
0.69	2.17 * (45.8)	10.75 * (29.5)	29.33 * (56.2)
1.1[a]	1.00 * (75.0)	7.83 * (48.6)	17.08 * (74.5)

Values in parentheses represent percent inhibition compared to solvent control

Negative values indicate a higher yield in treatment group compared to solvent control

* Statistically significantly different compared to control ($p \leq 0.05$, Williams t-test)

[a] Non-centrifuged sample

Table 104: Growth rate of Scenedesmus subcapitatus after exposure to tolclofos-methyl

Mean measured concentration [mg a.s./L]	Growth rate [x 10^4 cells/mL/d] (percent inhibition)		
	24 h	48 h	72 h
Solvent control	1.57	1.39	1.40
0.13	1.56 (0.8)	1.55 (-11.4)	1.34 (4.2)
0.22	1.42 (9.3)	1.47 (-6.0)	1.32 * (5.9)
0.39	1.36 (13.5)	1.30 (6.5)	1.23 * (12.0)
0.69	1.13 * (28.3)	1.23 (11.6)	1.14 * (19.0)
1.1[a]	0.672 * (57.2)	1.07 * (23.2)	0.961 * (31.4)

Values in parentheses represent percent inhibition compared to solvent control

Negative values indicate a higher yield in treatment group compared to solvent control

* Statistically significantly different compared to control ($p \leq 0.05$, Williams t-test)

[a] Non-centrifuged sample

The reported effect concentrations are presented in the table below.

Table 105: Effect concentrations reported in the original study (Sayers, 2003) and the re-calculated effect concentrations based on geomean measured values.

	Endpoints as presented in original report (Sayers, 2003) [mg a.i./L]	Re-calculated endpoints [mg a.i./L]
Yield		
EC_{10}	n.r.	0.10
EC_{20}	n.r.	0.18
EC_{50}	n.r.	0.49
NOEC	n.r.	0.12
Growth rate		
EC_{10}	n.r.	0.34
EC_{20}	n.r.	0.67
EC_{50}	> 1.1 (estimated)	0.90*

	Endpoints as presented in original report (Sayers, 2003) [mg a.i./L]	Re-calculated endpoints [mg a.i./L]
NOEC	0.22	0.12

n.r. = not reported

* could not be determined due to mathematical reasons and was therefore estimated to be greater than the highest test concentration

Conclusion

The laboratory study with toxicity-testing of *Scenedesmus subspicatus* was accepted without any further comments in DAR (2003) and both parts of this study are well performed and reported. However, since there are new validity criteria for toxicity testing with algae (OECD TG 201, 2011), the applicant has conducted new calculations based on the existing data.

Even though the initial biomass concentration is higher than recommended in the standard (1.0×10^4 cells/mL, compared to the recommended $2\text{-}5 \times 10^3$ cells/mL), the validity criteria were fulfilled according to OECD TG 201 (2011) with an average specific growth rate >0.92 day-1. Furthermore, the coefficient of variation of average growth rate was <7% for 0-72 h and <35% for the section-by-section specific growth rate.

Table 106: 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 (%)
Control	1					1.33	0.15	0.12		
	2	1.34	0.017	0.012	1.25	1.30	0.26	0.20	0.219	21.9
	3					1.33	0.45	0.34		
Solvent control	1					1.40	0.44	0.31		
	2	1.40	0.068	0.048	4.84	1.33	0.12	0.087	0.210	21.0
	3					1.50	0.35	0.23		

According to OECD TG 23 (2000), reported effect concentrations cannot be higher than saturation concentration. Based on the measured concentration at 0 h in the centrifuged sample, the correct E_rC_{50} for growth rate should therefore be >0.7 mg a.i./L. The value of this growth rate dcn however not be used for classification purpose. Instead the EC_{50} of biomass will be used.

Overall it can be summarized that tolclofos-methyl is very toxic to the algae *Scenedesmus subspicatus* with a 72 h EC_{50} (biomass)=0.49 mg/L and NOEC =0.12 mg a.s/L.

11.6.4 Chronic toxicity to other aquatic organisms

Reference:	Putt, A.E. (2002) Tolclofos-methyl – The Full Life-Cycle Toxicity To Midge (*Chironomus riparius*) Under Static Conditions Wirzinger, G, Ruhnke, H (2016) Re-calculation of toxicity endpoints for effects of Tolclofos-methyl on *Chironomus riparius*
Company Report No.:	QW-0063 13048.6331 (994-08023) QW-0162
Guideline:	OECD Draft TG 219 (2001)
GLP:	Yes
Previous evaluation:	In DAR (2003)
Material and methods:	
Test material:	Tolclofos-methyl (non-radiolabelled) [^{14}C]Tolclofos-methyl
Lot/Batch No:	90437G (non-radiolabelled) CP-2427
Purity:	98.0% (non-radiolabelled) 98.9%
Species:	*Chironomus riparius*
Test system/test conditions:	The test was performed in a static system for 28 days with eight replicates per concentration, four for monitoring biological results and four for determining exposure concentration in the overlying water. The total volume in the test vessels was 375 mL with a ratio of sediment:water of 1:4. Each replicate had 20 midge larvae (3 days old). Midge emergence; abnormal behaviour; sex and number of adult midges that emerged daily were recorded. The development rate was also determined. The test substance was applied to the water phase. Nominal test concentrations in the water phase were: 0 (control), 0 (solvent control), 0.063, 0.13, 0.25, 0.5, 1.0 mg a.i./L. Temperature: 19-20°C; pH: 5.5-7.7; dissolved oxygen: 7.6-9.6 mg/L (60% of air saturation at 20°C = 5.4 mg/L); photoperiod: 16 hours light (970 – 1080 lux): 8 hours dark. Overlying water and sediment in all test levels were analysed at each interval (day 0, 7, 14 and 28) for total ^{14}C by LSC. The pore water samples in the 0.063, 0.25 and 1.0 mg a.s./L treatment levels were analysed at each interval for total ^{14}C by LSC (LOQ = 0.00468 mg a.s./L, 0.018 mg a.i./kg). In addition, the 0.063, 0.25 and 1.0 mg a.s./L treatment levels were analysed at each interval for the concentration of tolclofos-methyl in water and sediment by high performance liquid chromatography using radiochemical detection (HPLC/RAM). A DM-TM (metabolite) reference standard was analysed by HPLC/UV on three occasions to establish its retention time. ECx values were calculated with regression analysis.

Results:

Measured concentrations corresponded to 7.6-16% of the nominal concentrations in the overlying water and 31-84% of the nominal concentrations in the sediment at the end of the test. The results of the HPLC/RAM analysis established that any test substance that remained in the water column during the study degraded to DM-TM (0 to 9 % of applied ^{14}C) or other minor metabolites (0 to <7% of applied ^{14}C) by the end of the 28-day exposure. The test substance that portioned to the sediment generally remained as parent compound.

Table 107: Concentrations of total [^{14}C] residue measured by liquid scintillation counting (LSC) in overlying water and sediment samples during the 28-day exposure of *Chironomus riparius* to tolclofos-methyl

Nominal Concentration (mg a.s./L)	Measured Concentration, mg a.s./L (mg a.s./kg for sediment) (% of nominal concentration in water)			
	1-Hour	Day 7	Day 14	Day 28
Overlying water				
Control	<LOQ	<LOQ	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ
0.063	0.064 (100%)	0.0067 (11%)	0.0074 (12%)	<LOQ
0.13	0.13 (100%)	0.022 (17%)	0.019 (15%)	0.0099 (7.6%)
0.25	0.23 (94%)	0.035 (14%)	0.031 (12%)	0.019 (7.6%)
0.50	0.47 (94%)	0.11 (22%)	0.10 (20%)	0.070 (14%)
1.0	0.76 (76%)	0.16 (15%)	0.12 (12%)	0.16 (16%)
Sediment (% of applied ^{14}C)				
Control	<LOQ	<LOQ	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ	<LOQ	<LOQ
0.063	0.020 (15%)	0.056 (42%)	0.071 (54%)	0.075 (57%)
0.13	0.024 (8.8%)	0.22 (81%)	0.20 (73%)	0.13 (48%)
0.25	0.063 (12%)	0.34 (65%)	0.18 (34%)	0.16 (31%)
0.50	0.10 (10%	0.72 (69%)	0.86 (82%)	0.88 (84%)
1.0	0.26 (12%)	1.5 (72%)	1.2 (57%)	0.87 (41%)

Table 108: Mean percentage emergence and mean development rates calculated at test termination (day 28) of the midge (*Chironomus riparius*) full life-cycle exposure with tolclofos-methyl

Nominal concentration (mg a.s./L)	Mean Percent Emerged	Mean Development Rates		
		Males	Females	Males/Females (pooled data)
Control	86	0.0649	0.0553	0.0597
Solvent control	85	0.0664	0.0555	0.0600
Pooled control	86	0.0657	0.0554	0.0599
0.063	86	0.0654	0.0570	0.0613
0.13	86	0.0630	0.0558	0.0593
0.25	78	0.0615	0.0538	0.0577
0.50	78	0.0592	0.0528	0.0561*
1.0	79	0.0521	0.0481	0.0501*

* significantly different from the pooled control data (Williams' test, $p < 0.05$)

The mean percentage emergence in the control after 28 days was 85%. No toxic effects on emergence were observed. The two highest concentrations had significantly lower development rate than the control. Based

on the nominal concentrations NOEC for development rate was determined to 0.25 mg a.i./L, EC_{10} 0.62 (95% CI 0.45-0.87) and EC_{20} and EC_{50} to >1 mg a../L, based on nominal concentrations.

Conclusion

The effect values reported are based on nominal concentrations. However, according to OECD TG 219 (2004), effect concentrations should be calculated based on the initially measured concentration in the water phase. The RMS therefore suggests to adjust the effect value to the measured concentrations after 1 hour. The NOEC would then be 0.23 mg a.s./L and 0.063 mg a.s./kg sediment for water and sediment, respectively. The EC_{20} and EC_{50} values would be >0.76 mg a.s/L and >0.26 mg a.s./kg sediment for water and sediment, respectively. The reported EC_{10} was based on nominal concentration, however, this value was higher than the NOEC.

In can be cocluded that tolclofos-methyl is very toxic towards sediment dwelling organisms like *Chironomus riparius* with a 28 day NOEC=0.25 mg/L.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Tolclofos-methyl fulfils the classification criteria for Aquatic Acute 1, since its toxicity to aquatic organisms from all three trophic levels (fish,crustacean and algae) is below 1 mg/l (EC_{50}< 1 mg/l).

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Tolclofos-methyl fulfils the criteria for classification as Aquatic Chronic 1 since its chronic toxicity to aquatic species from two throphic level is below 1 mg/l (fish as the most sensitive species *Oncorhynchus mykiss* (Rainbow Trout) NOEC (97 days)=0.012 mg/l. and the invertebrate *Daphnia magna* 21 day NOEC=0.026 mg/L and the acute 72 h NOEC =0.12 mg a.s/L e of algae *Scenedesmus subspicatus* (since there was no chronic toxicity data for algae) also below 1 mg/l and combined with that the substance is not rapidly biodegradable and has a high bioaccumulation potential.

Based on log Kow=3.8 and BCF=670 in fish, tolclofos-methyl is considered to possess the potential to bioconcentrate for classification purposes, as indicated by the experimentally determined BCF in fish of >=500.

For classification purpose it is applicable to classify tolclofos-methyl as not readily degradable (<70% degradation within 28 days) according to the ready biodegradable test OECD 301C that showed after 7 days -2 percantage degradation and after 14 days -1 percantage degradation (BOD-B/TODx100%) was found in the active sludge.

In one water-sediment studies with two test systems it also was shown that tolclofos-methyl was not rapidly degradable with DT_{50}= 32-64 days (the CLP citeria if DT_{50}<16 days the substance undergoes fast degradation).

Also the abiotic degradation showed that hydrolysis of tolclofos-methyl is stable with a DT_{50} that varies between 97 -126 days depending on pH and temperatures.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Tolclofos-methyl can be classified as Aquatic Acute 1, with a M-factor 1 (0.1<L(E)C_{50} <=1 mg/L) based on acute toxicity of invertebrate *Americamysis bahia* 96 h LC_{50} =0.377 mg a.s./L.

Tolclofos-methyl can be classified as Aquatic Chronic 1 with an M-factor 1 (0.01<NOEC=<) based on NOEC for growth for fish *Oncorhynchus mykiss* 97 days=0.012 mg/l and that the substance is not rapidly biodegradable and has a high bioaccumulation potential.

Hazard statement codes: *Hazardous to the aquatic environment*

Aquatic Acute 1; H400, M-factor 1

Aquatic Chronic 1; H410, M-factor 1

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Tolclofos-methyl (ISO) or *O*-(2,6-dichloro-p-tolyl) *O,O*-dimethyl thiophosphate is an active substance used in plant protection products. It is used as a contact fungicide for the control of *Rhizoctonia*. The representative uses for the renewal of approval of tolclofos-methyl includes potatoes, lettuce and ornamentals.

Tolclofos-methyl has for environmental hazards a current Annex VI entry with a harmonised classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) but with no M-factors specified.

The dossier submitter proposed to add the M-factor of 1 for both Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

Degradation

The dossier submitter proposed to consider tolclofos-methyl as <u>not</u> rapidly degradable for classification purposes. The basis for this proposal is a non-GLP OECD TG 301 C study (Nambu, 1984) with reliability of 1 that found after 7 and 14 days no degradation in the active sludge and a GLP EEC Method C.7 guideline hydrolysis study (Lewis, 2001b) with reliability of 1 found that tolclofos-methyl is not prone to hydrolysis under environmental conditions.

Tolclofos-methyl has a vapour pressure of 8.77 x 10^{-4} Pa at 20ºC and a relatively low water solubility and therefore the value of Henry's law constant (calculated as vapour pressure x mol. weight x water sol.-1) is relatively high at 0.37 Pa m3 mol-1 at 20°C. The substance therefore has a tendency to volatilise from water and moist surfaces. Consequently, degradation testing on mineralisation in water is hampered by the volatile nature of the test substance.

Two water/sediment test systems, one GLP OECD TG No 309 study (Adam, 2015) with reliability of only 3 and one GLP SETAC, 1995 guideline study (Lewis, 2001c, re-assessed by Weimann & Lobe, 2015c) with reliability of 1 are available. In the second study, a $DT_{50whole\ system}$ (20°C) between 32.2 and 64.1 days was measured. Both studies discovered only limited mineralisation and thus support the conclusion that tolclofos-methyl is not rapidly degradable for classification purposes.

Aquatic Bioaccumulation

The dossier submitter proposed to consider tolclofos-methyl as having a high bioaccumulation potential in the aquatic environment for classification purposes. The basis for this proposal is a log Pow of 3.8 and two studies with measured BCF-values. A total-C_{14}-BCF whole fish value of 670 (on day 28, which is also equal to the mean value from steady state at 7 days until the end of the study) was measured by Anonymous (1986) and a total-C_{14}-BCF value of 506 (steady state value obtained from the lower test concentration) was measured by Anonymous (2004). In the first BCF study, the parent substance accounted for around 80 % of the C_{14} measurement so the BCF could be adjusted though it would still be (just) above 500 L/kg. Ideally it would be lipid-normalised, but there is no information to allow that. The second BCF was lipid-normalised, and the parent substance BCF is below 500 L/kg. Metabolism is usually considered to be a depuration mechanism. However, since no information is available on the toxicity of the metabolites observed in the BCF studies, the total-C_{14}-BCF values are the most appropriate. Overall, it can be concluded that tolclofos-methyl BCF in fish is 670, which is above the CLP criteria BCF for fish ≥500 and can, therefore, be considered bioaccumulative for classification purposes.

Acute Aquatic Toxicity

The dossier submitter proposed to classify tolclofos-methyl as Aquatic Acute 1 (H400) for the aquatic environment with an M-factor of 1. The basis for this proposal is that the toxicity to aquatic organisms from all three trophic levels (fish, crustacean and algae) is in the range 0.1 – 1 mg/L. Tolclofos-methyl is very toxic with the most sensitive species the saltwater mysid *Americamysis bahia* with 96h EC_{50} =0.377 mg/L The aquatic metabolite tested (DM-TM) was of lower toxicity relative to parent at all aquatic trophic levels (fish, daphnia, green algae).

Table*: Summary of acute aquatic toxicity of tolclofos-methyl*

Method	Species	Results[1]	Remarks	Reference
FIFRA Guideline 72-1, OPPTS 850.1075, OECD TG 203, EC Guideline Annex V - Method C.1.	*Oncorhynchus mykiss* Rainbow Trout	Acute 96 hr (flow-through), LC_{50}=0.69 mg a.s./L (mm)	Reliability 1	Anonymous (2003) QW-0071
FIFRA Guideline 72-1, OPPTS 850.1075, OECD TG 203, EC Guideline Annex V - Method C.1.	*Lepomis macrochirus* Bluegill sunfish	Acute 96 hr (flow-through), LC_{50}>0.720 mg a.s./L (mm)	Reliability 1	Anonymous (1989) QW-91-0036
EPA FIFRA, 40 CFR, Part 158.145, Guideline 72-2	*Daphnia magna* Water flea	Acute 48 h (static) 48 mg a.s./L (mm)	Reliability 1	Murrell, H. *et al*. (1994) QW-41-0046
OPPTS 850.1035 U.S EPA Guideline	*Americamysis bahia* Saltwater mysid	96 h (semi-static LC_{50})= 0.377 mg a.s./L (mm)	Reliability 1	Palmer, S.J. *et .al* (2010a) QW-0111
OECD No. 201, EC Guideline Annex V - Method C.3.	*Scenedesmus subspicatus*	72 h (static) EyC_{50}=0.49 mg a.s. /L	Reliability 1	Sayers, L.E. (2003) QW-0072 and Wirzinger,G *et*

OECD TG 201 (2011) (for the re-calculations)		EC_{50} (growth) no information) NOEC =0.12 mg a.s/L		*al.* (2014) QW-0144

Chronic Aquatic Toxicity

The dossier submitter proposed to classify tolclofos-methyl as Aquatic Chronic 1 (H410) with an M-factor of 1. The basis for this proposal is that tolclofos-methyl is a 'not rapidly degradable' substance, it is considered bioaccumulative, and that chronic toxicity to fish as the most sensitive species *Oncorhynchus mykiss* (Rainbow Trout) is the 97 days (flowthrough) NOEC (growth) of 0.012 mg/l (with EC_{10} of 0.013 mg/L).

***Table**: Summary of chronic aquatic toxicity of tolclofos-methyl*

Method	Species	Results[1]	Remarks	Reference
U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4	*Oncorhynchus mykiss* Rainbow Trout	Chronic (flow-through, 97 days) Growth, NOEC =0.012 mg a.s./L EC_{10} =0.013 mg a.s./L	Reliability 1	Anonymous (1991) QW-11-0040; Wirzinger and Ruhnke (2016) QW-0163
U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4	*Daphnia magna* Water flea	21 d(flowthrough) Reproduction NOEC=0.026 mg a.s./L EC_{10}=0.036 mg a.s./L (mm)	Reliability 1	Burgess, D (1989) QW-91-0031; Wirzinger and Ruhnke (2016) QW-0159
OECD Draft TG 219 (2001)	*Chironomus riparius* Midget larvae	28 d (static water/sediment system) Development NOEC=0.25 mg/l EC_{10}=0.62 mg a.s./L	Reliability 1	Putt, A.E. (2002) QW-0063; Wirzinger and Ruhnke (2016), QW-0162
OECD No. 201, EC Guideline Annex V - Method C.3. OECD TG 201 (2011) (for the re-calculations)	*Scenedesmus subspicatus*	72 h (static) EyC_{50}=0.49 mg a.s. /L EC_{50} (growth) no information) NOEC =0.12 mg a.s/L	Reliability 1	Sayers, L.E. (2003) QW-0072 and Wirzinger,G *et al.* (2014) QW-0144

Further, the 21 day NOEC (reproduction) of 0.026 mg/L for the invertebrate *Daphnia magna* and the 72 h NOEC (yield) of 0.12 mg a.s/L of the algae *Scenedesmus subspicatus* support the same classification as Aquatic Chronic 1 (H410) with an M-factor of 1.

Comments received during public consultation

The public consultation received two comments from MSCAs and one comment from industry on the proposals for environmental classification. All three agreed with the proposed classification for tolclofos-methyl as Aquatic Acute 1 (H400) with an M-factor of 1 and as Aquatic Chronic 1 (H410) with an M-factors of 1.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal of the dossier submitter to consider tolclofos-methyl as not rapidly degradable for classification purposes, as indicated in the ready biodegradability and the two water/sediment tests.

Aquatic Bioaccumulation

RAC agrees with the proposal of the dossier submitter to consider tolclofos-methyl as being bioaccumulative for classification purposes, due to the measured BCF value in fish of 670.

Acute Aquatic Toxicity

RAC agrees with the proposal of the dossier submitter to classify tolclofos-methyl as **Aquatic Acute 1 (H400) with an M-factor of 1**, based on the acute toxicity of invertebrate *Americamysis bahia* (96h LC_{50} =0.377 mg/L). The M-factor of 1 is appropriate because the LC_{50}/ EC_{50}values for all three trophic levels are in the range 0.1 – 1 mg/L.

Chronic Aquatic Toxicity

RAC notes that there is no chronic NOEC for the most acutely sensitive species the saltwater mysid *Americamysis bahia* (mysid shrimp).

Based on the available chronic toxicity data, RAC agrees to classify tolclofos-methyl as Aquatic Chronic 1, as the 97d NOEC for growth for *Oncorhynchus mykiss* was 0.012 mg/L. **The corresponding M factor is 1**, as the toxicity falls within the range 0,01 < NOEC ≤ 0,1 and the substance is considered not rapidly degradable for classification purposes.

It should be noted that RAC also applied the surrogate approach, which results in the same chronic classification as proposed by the dossier submitter based on the available NOECs.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Hazard class not assessed in this dossier.

13 ADDITIONAL LABELLING

None

14 REFERENCES

See separate Annexes.

15 ANNEXES

Annex I Physical and Chemical properties

Annex II Toxicology and metabolism

Annex III Environmental Fate and Behaviour

Annex IV Ecotoxicology

CONFIDENTIAL Annex V Information on tested formulations