Committee for Risk Assessment

RAC

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

Adopted

15 March 2019

15 March 2019

CLH-O-0000001412-86-266/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **260-515-3**

CAS Number: **57018-04-9**

The proposal was submitted by **Sweden** and received by RAC on **11 June 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **18 July 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 September 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Marja Pronk**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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 Aquatic Chronic 1	**Retain** H317 H400 H410	**Retain** GHS09 GHS07 Wng	**Retain** H317 H410		**Add** M=1 (acute) M=1 (chronic)	
RAC opinion	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 Aquatic 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 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 Aquatic Chronic 1	H317 H400 H410	GHS09 GHS07 Wng	H317 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

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*. It has an existing entry in Annex VI of the CLP Regulation and 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).

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.

HUMAN HEALTH HAZARD EVALUATION

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.

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)**.

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**.

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**.

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**.

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	0, **200, 600** and 2000 ppm equal to:	Liver NOAEL m/f: 600 ppm	200 ppm: None 600 ppm:

Study	Dose levels	Target organ(s) NOAEL	Effects at relevant doses for classification
No GLP (Anon. 1979, RAR Vol.3 B.6.3.3.2/01)	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		- 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 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	<u>Main phase</u> 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	NOAEL m/f: 1800 ppm	Main study: <u>300 ppm:</u> None <u>1800 ppm*:</u> None Satellite study: <u>300 ppm:</u>

Study	Dose levels	Target organ(s) NOAEL	Effects at relevant doses for classification
GLP (Anon. 2007, RAR Vol.3 B.6.7.1.2/01)	Satellite group 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		↓ erythrocyte cholinesterase (f: 8% at week 14) 1800 ppm*: ↓ 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	100 ppm None 300 ppm: 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	100 ppm: None 300 ppm: 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	10 ppm: None 50 ppm: None
DERMAL			
21-day (6 h/d, 5 d/wk, 3 wks) Rabbit, New Zealand White/Hazleton Dutchland (5/sex/dose)	0, **30**, **300** and **1000** mg/kg bw/d vehicle: Acetone	Skin, kidney NOAEL(systemic) m: ≥1000 mg/kg bw/d	30 mg/kg bw/d: - dermal irritation (erythema (m, f)) - histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration (m, f))

Study	Dose levels	Target organ(s) NOAEL	Effects at relevant doses for classification
EPA Guideline 158, 82-2 GLP (Anon. 1986, RAR Vol.3 B.6.3.6/01)	Guidance value for classification **≤ 1200** mg/kg bw/d	NOAEL(systemic) f: 300 mg/kg bw/d NOAEL (local) m/f: <30 mg/kg bw/d	300 mg/kg bw/day: - dermal irritation (erythema (m, f)) ↓plasma cholinesterase activity (f: 29%) - histopathological changes in the skin (hyperkeratosis, acanthosis, subepidermal pleocellular infiltration (m, f)) 1000 mg/kg bw/day: - 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.

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**.

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**.

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**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards

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 not 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	*Americamysis bahia*	96 h (semi-static LC_{50})=	Reliability 1	Palmer, S.J. *et .al* (2010a) QW-0111

U.S EPA Guideline	Saltwater mysid	0.377 mg a.s./L (mm)		
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

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 $\leqslant$ 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).