Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

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

CLH-O-0000001412-86-281/F

Adopted

15 March 2019

15 March 2019

CLH-O-0000001412-86-281/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: **thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)**

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

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

The proposal was submitted by **Sweden** and received by RAC on **3 April 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 **23 April 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 June 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Gabriele Aquilina**

Co-Rapporteur, appointed by RAC: **Ignacio De La Flor Tejero**

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	006-069-00-3	thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)	245-740-7	23564-05-8	Acute Tox. 4* Skin Sens. 1 Muta. 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H317 H341 H400 H410	GHS08 GHS07 GHS09 Wng	H332 H317 H341 H410			
Dossier submitters proposal	006-069-00-3	thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)	245-740-7	23564-05-8	**Add** STOT RE 2 **Modify** Muta. 1B Acute Tox. 4 **Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**Add** H373 **Modify** H340 **Retain** H332 H317 H400 H410	**Retain** GHS08 GHS07 GHS09 **Modify** Dgr	**Add** H373 **Modify** H340 **Retain** H332 H317 H410		**Add** inhalation: ATE = 1.7 mg/L (dusts and mists) M=10 M=10	
RAC opinion	006-069-00-3	thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)	245-740-7	23564-05-8	**Retain** Muta. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2 **Modify** Acute Tox. 4	**Retain** H341 H332 H317 H400 H410 **Add** H351	**Retain** GHS08 GHS07 GHS09 **Modify** Wng	**Retain** H341 H332 H317 H410 **Add** H351		**Add** inhalation: ATE = 1.7 mg/L (dusts and mists) M=10 M=10	
Resulting Annex VI entry if agreed by COM	006-069-00-3	thiophanate-methyl (ISO); dimethyl (1,2-phenylenedicarbamothioyl)biscarbamate; dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)	245-740-7	23564-05-8	Carc. 2 Muta. 2 Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H341 H332 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H351 H341 H332 H317 H410		inhalation: ATE = 1.7 mg/L (dusts and mists) M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

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

PHYSICAL HAZARD EVALUATION

RAC evaluation of explosives

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

RAC agrees that no classification as explosives is warranted. The data presented is conclusive but not sufficient for classification.

RAC evaluation of flammable solids

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

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

RAC evaluation of self-reacting substances

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

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

RAC evaluation of pyrophoric solids

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

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

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

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

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

RAC evaluation of oxidising solids

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

One MSCA agreed to no classification for physical hazards.

Assessment and comparison with the classification criteria

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

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

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

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

Comments received during public consultation

No specific comment on acute toxicity oral was received.

Assessment and comparison with the classification criteria

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

Summary of the Dossier Submitter's proposal

Acute toxicity: dermal

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

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

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

Comments received during public consultation

No specific comment on acute toxicity dermal was received.

Assessment and comparison with the classification criteria

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

Summary of the Dossier Submitter's proposal

Acute toxicity: inhalation

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

Five Crj:CD(SD) rats/sex/group were exposed to a dust aerosol (MMAD: 3.7-4.5 µm) of thiophanate-methyl at concentrations of 0.5 mg/L (females only), 1.0, 1.5, 1.6 and 1.9 mg/L, or to clean air (control group), for 4 hours, and then observed for 14 days. Exposure was via whole body inhalation. During the study period, 5/5 males and 3/5 females died at 1.9 mg/L, and 1/5 females died at 1.0 mg/L. The calculated 4-hour ATE values were 1.7 mg/L for males and 1.9 mg/L for females. Several clinical signs were observed (e.g. tremors, convulsions, decreased motor activity), but at doses close to the LC_{50} values.

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

Summary of the Dossier Submitter's proposal

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

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

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

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

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

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

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

The DS proposed no classification for STOT SE.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

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

Categories 1 and 2

In the acute toxicity studies performed, no systemic effects were noted after oral and dermal administration. After inhalation exposure, clinical signs such as tremors, convulsions, low sensitivity, decreased motor activity, ptosis, ataxia and ventral position were observed at doses close to the LC_{50} value. According to the CLP guidance, care should be taken not to assign a "double classification" for the same effect and as these effects occurred close to the lethal doses, they are considered to have been unspecific effects of acute toxicity and are therefore not considered to justify classification in STOT SE. No adverse effects were noted in the acute neurotoxicity study (see the section on STOT RE for details). In the one-year dog study, tremors were seen shortly after dosing in a dose-response related manner during the initial three weeks of study but were not observed subsequently. No tremors were observed in a 90-day study in the same species and at the same dose levels. The significance of the finding is unclear and in DS's opinion did not justify classification in STOT SE. There were no relevant early effects noted in the other repeated dose studies.

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

Category 3

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

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of serious eye damage/eye irritation

Summary of the Dossier Submitter's proposal

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

In this specific case, for thiophanate-methyl, there is a very high response rate following the use of a high intradermal induction dose. It is therefore possible that the use of a lower dose would

result in a response rate which fulfils the criteria for sub-category 1A. Sub-categorisation of thiophanate-methyl is therefore not proposed.

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

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

Summary of the Dossier Submitter's proposal

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

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

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

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

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

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

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

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

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

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

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

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

Classification in STOT RE 2 was proposed by DS based on the mortalities seen in rabbits at 125, 300 and 600 mg/kg bw/d in a developmental toxicity study with 14 days exposure to

thiophanate-methyl, as well as on thyroid effects (hypertrophy, hyperplasia and increased organ weight) in rats, supported by similar findings in dogs. The following hazard statement was proposed: H373 – May cause damage to organs through prolonged or repeated exposure.

Comments received during public consultation

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

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

The comment against the classification was based on that, even if the thyroid size is increased in a clearly dose dependent manner at or above the guidance value for STOT RE 2, a mere increase in organ size (hypertrophy/hyperplasia) is not an adverse effect sufficient to warrant classification for STOT RE. Moreover this MSCA stated that the criteria allow classification when a relevant mechanism is demonstrated or relevant secondary effects or relevant organ dysfunctions are demonstrated. However in this case, thiophanate-methyl inhibits TPO resulting in reduced thyroid hormone levels that has an effect on thyroid size. It is thus considered by the MSCA that the thyroid size is a result from thyroid hormone inhibition and that a mere increased thyroid size can be seen as a successful adaptive mechanism without further toxicological relevant consequence. Notably, the mortality observed starting at 150 mg/kg bw/d in the rabbit developmental toxicity range finding study (dosed for 14 days) does not seem as relevant for classification for STOT RE due to low mortality incidence without a clear dose/time-dependent relationship. Due to the short duration of developmental tests, the MSCA also states that it is unclear whether this should be considered acute toxicity or repeated dose toxicity.

Assessment and comparison with the classification criteria

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

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

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

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

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

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

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

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

Study	Species	Dose	Thyroid effects
		(M: 54.4 mg/kg bw/d; F: 63.5 mg/kg bw/d)	M, F: hypertrophy/hyperplasia
		6 000 ppm (M: 280.6 mg/kg bw/d; F: 334.7 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy/hyperplasia, adenoma
	Dog 1 year RAR Vol 3, B.6.3.2.5	Guidance value STOT RE 2: 25 mg/kg bw/d	
		8 mg/kg bw/d	F: Weight↑
		40 mg/kg bw/d	M, F: Weight↑ F: Hypertrophy, (M: T4↓, interim: 6 m)
		200 mg/kg bw/d	M, F: Weight↑ M, F: Hypertrophy; hyperplasia (2 animals) (M: T4↓, interim 6 m and 12 m)
	Mouse 18-m RAR Vol 3, B.6.5.3	Guidance value STOT RE 2: 16.7 mg/kg bw/d	
		150 ppm (M: 23.7 mg/kg bw/d, F: 28.7 mg/kg bw/d)	No effects
		640 ppm (M: 98.6 mg/kg bw/d, F: 123.3 mg/kg bw/d)	No effects
		3 000 ppm (M: 467.6 mg/kg bw/d, F: 557.9 mg/kg bw/d)	M: Weight ↑ TSH ↑ at 9 m but not at 18 m
		7 000 ppm (M: 1 078.8 mg/kg bw/d, F: 1 329.4 mg/kg bw/d)	M, F: Weight ↑ TSH ↑ at 9 m but not at 18 m F. T4↓ at 9 m but not at 18 m
Mechanism study (*in vivo*)	Rat RAR Vol 3, B.6.5.1	6 000 ppm Male	Weight ↑ T3, T4↓, TSH↑
2-gen study	Rat RAR Vol 3, B.6.6.1.1	Guidance value STOT RE 2: 40 mg/kg bw/d	
		200 ppm (M: 14.6 mg/kg bw/d, F: 18.0 mg/kg bw/d)	**(F: Weight) M : hypertrophy /hyperplasia**
		630 ppm (M: 46.0 mg/kg bw/d, F: 55.4 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy/hyperplasia
		2 000 ppm (M: 147.1 mg/kg bw/d, F: 172.9 mg/kg bw/d)	M, F: Weight↑ M, F: hypertrophy/hyperplasia M, F: TSH↑, M: T4↓
90-day neurotoxicity study	Rat RAR Vol 3, B.6.7.1.2	Guidance value STOT RE 2: 100 mg/kg bw/d	
		100 ppm (M: 6.2 mg/kg bw/d, F: 6.8 mg/kg bw/d)	No effects (histopathology and hormones not investigated)
		500 ppm (M: 30.3 mg/kg bw/d, F: 34.9 mg/kg bw/d)	
		2 500 ppm (M: 149.6 mg/kg bw/d, F: 166.3 mg/kg bw/d)	M, F: Weight↑ (histopathology and

Study	Species	Dose	Thyroid effects
			hormones not investigated)

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

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

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

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

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

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

from the proportion observed after exposure to the known aneugen carbendazim. The DS considered this result as an evidence of the clastogenic potential of thiophanate-methyl.

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

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

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

A dominant lethal test performed as part of a mutagenic, cytogenetic and teratogenic study gave no indication of an increase in chromosome aberrations. Neither was there an indication of treatment-related dominant lethal mutations in male mice. However, the study suffered from many deficiencies including the lack of reporting of individual data and the absence of a positive control to verify appropriate functioning of the test. Thus, based on this result, it was difficult to exclude that the aneugenic effects of thiophanate-methyl observed in somatic cells would not occur in germ cells and overall the result was considered inconclusive. Moreover, although the dominant lethal assay is recommended as a method to follow up positive results observed in *in vivo* genotoxicity tests in somatic cells, the DS considered that the test is generally regarded to have low sensitivity and this is also recognised in the OECD TG 478. Furthermore, it is noted that carbendazim, which is concluded to be a germ-cell mutagen (currently classified Muta. 1B, see section on classification) was also negative in dominant lethal tests (DAR on carbendazim, 2009).

Additional data were requested by EFSA during the peer review and consisted of four new studies testing mutagenic effects of thiophanate-methyl in germ cells of mice, one spermatogonial chromosome aberration study with a preceding pre-test and a micronucleus study in germ cells with a preceding pre-test. All four studies were concluded to be negative by the study author, since no statistically significant increase or dose-dependent relationship were observed in

chromosome aberrations or micronuclei. However, the DS observed that no data demonstrating target-cell exposure were presented and, therefore, the negative results of the studies have low reliability. The reliability can be further questioned by the arguments presented above for the new bone marrow micronucleus test by (RAR Vol 3, B.6.4.2.2 Doc. No. 557-016), i.e. that the strain Crl:CD-1 (ICR) was used and that there is reason to be concerned about the sensitivity of this strain to detect mutagenic effects of thiophanate-methyl.

Conclusion by the DS:

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

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

-positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells".

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

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

Considerations on structure-activity relationship

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

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

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

Considerations on threshold

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

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

Modified from Koroiwa, 2018

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

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

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

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

Conclusions

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

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

The substance is systemically available and was detected in gonads of mice at concentrations close to those found to cause aneugenicity *in vitro*. On the other hand, the available *in vivo* studies in germ cells did not demonstrate any genotoxic effect. Taking into account all available

data in a weight of evidence approach, RAC is of the view that criteria for classification in Category 1B are not met.

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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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

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

Table: *Thyroid changes*

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

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

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

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

- Measurement of T3, T4 and TSH, drug intake, liver and thyroid weights and total cholesterol in serum. Measurements of enzyme induction: cytochrome P450, cytochrome

b5, NADPH-cytochrome c reductase, UDP-glucuronosyltransferase) and microsomal protein (rat).
• Administration of thiophanate-methyl followed by daily T4 supplementation, measurements of body weight, thyroid and liver weights and total cholesterol levels (rat).
• Thyroid peroxidase (TPO) activity measurements (pig) using PTU as a reference substance.
• Proliferation of liver cells (mice and rats) using PB as a reference substance.

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

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

Table: *Incidence of mouse hepatocellular adenomas*

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

**p < 0.01
a outside historical control range (HCD: Males: 0-16.3 % (mean 8.2 %); Females: 0-2.7 % (mean 1.4 %))

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

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

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

1. Long-term toxicity and carcinogenicity study in rats (B6.5.2 in the RAR). In this study the number of parameters examined (clinical chemistry and haematological examination) was not as extensive as required by the OECD TG 452, but it is considered to provide useful

supportive information on the toxicological profile of the test substance. In particular histopathological changes in the thyroid (follicular epithelium hypertrophy, decrease of colloidal substance) and in the testes (reduction in spermatogenesis) were reported. It is also noted that the dose range used was lower than in the study reported above (B.6.5.1 in the RAR).

2. Long-term toxicity and carcinogenicity study in Beagle dogs 24 months (B.6.5.5 in the RAR) where some deviations from OECD TG 452 (version 1982). A moderate hypertrophy of the thyroid was noted in males and females at 50 and 250 mg/kg bw/d based on organ weight and histopathological examination. No effects on PBI (Protein binding iodine) levels were reported. Although no effect on carcinogenesis was observed, it is noted that the duration of the study (24 months) if compared to the lifespan of the dog was much shorter than the duration of the rodent study.
3. Chronic (1 year) oral toxicity study in the dog via capsule administration with thiophanate-methyl (B.6.3.2.5 in the RAR). Increased thyroid weight in females at lowest dose and at higher dose level a decrease of T4 in males and histopathological changes in the thyroid (hypertrophy and hyperplasia) were observed. No effect on carcinogenicity was reported. As noted for the study above, the duration of the study was not comparable to those applied to rodents.

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

Comments received during public consultation

Several comments were received in the public consultation. In particular, three MS supported the originally proposed classification as carcinogenicity cat 2 because also in line with the opinion expressed in the pesticide peer review process (EFSA 2018, doi: 10.2903/j.efsa.2018.5133, page 10). Moreover, one MS recalled that the substance has a structural alert for non-genotoxic carcinogenicity (thiocarbonyl) when put through the OECD toolbox and that there is a publicly available EPA report with information on the carcinogenicity of thiocarbonyl compounds. In this report the type of carcinogenicity observed is similar (predominantly thyroid hypertrophy/hyperplasia by inhibiting thyroid hormones resulting in tumours, but also liver tumours in mice as also observed for thiophanate-methyl), suggesting a possible similar carcinogenic mechanism. Two industries commented the carcinogenicity in PC, both expressing disagreement with the proposed classification. The principal elements discussed are: i) the rats tumours are observed beyond the MTD and ii) tumours observed in mice and rats are not relevant for humans. The DS agreed with industry for the first point, but disagreed with the argumentation regarding relevance to humans, where the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are considered secondary to liver enzyme induction and not relevant to humans. New mechanistic studies were provided by industry in PC where the thyroid peroxidase (TPO) inhibition demonstrated a lack of relevant inhibition for human TPO *in vitro* when tested up to precipitating concentrations.

Assessment and comparison with the classification criteria

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

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

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

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

(a) tumour type and background incidence;

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

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

(b) multi-site responses;

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

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

(c) progression of lesions to malignancy;

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

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

(d) reduced tumour latency;

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

cell adenocarcinomas were only observed at terminal sacrifice indicating slow tumour development.

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

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

Rats: Thyroid follicular cell adenomas statistically significant only in males.

Mice: Hepatocellular adenomas in both sexes.

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

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

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

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

(h) routes of exposure;

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

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

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

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

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

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

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

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

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

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

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

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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Reproductive toxicity

Adverse effects on sexual function and fertility

Two studies on fertility are available in rats.

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

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

Conclusion

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

Developmental toxicity

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

In the rat study (RAR Vol. 3 B.6.6.2.2.1), performed in accordance with GLP and OECD TG 414, no adverse effects on development were observed at doses up to 1 000 mg/kg bw/d. At this dose, there was a 22 % reduction in adjusted body weight gain. NOAEL for maternal toxicity was thus found to be 300 mg/kg bw/d.

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

The second rabbit study (RAR Vol. 3 B.6.6.2.2.3) was performed in accordance with GLP and OECD TG 414 and gave higher NOAEL values than the first rabbit study. NOAEL for developmental toxicity was 20 mg/kg bw/d based on supernumerary thoracic ribs at 40 mg/kg bw/d and NOAEL for maternal toxicity was 10 mg/kg bw/d based on minimal, transient but significant reduction in

maternal body weight gain and significantly reduced absolute and relative feed consumption values for the entire dosage period at 20 mg/kg bw/d. The 40 mg/kg bw/d dosage produced adverse clinical observations (reduced faecal output) and significantly reduced body weight gain and absolute and relative feed consumption values for the entire dosage period.

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

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

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

Conclusion

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

Lactation

Reduced pup weight was noted in the 2-generation study in rats. At 2 000 ppm, pup weights were reduced by up to 11 % (not statistically significant) in F1 with no effect on maternal body weight in the P generation. At the same dose level there was also a reduction in pup weight in F2a and F2b (up to 16 % reduction and statistically significant in F2b) but also reduced maternal

body weight gain F1 females (-12 % during gestation). At the mid dose of 630 ppm pup weight was reduced in F2b (m: -13 %; f: -16 %) with no corresponding effect on maternal body weight.

Conclusion

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

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

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

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

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

Fertility

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

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

Developmental toxicity

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

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

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

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

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

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

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

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

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

Lactation

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

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Thiophanate-methyl has an existing harmonised classification of Aquatic Acute 1 and Aquatic Chronic 1.

Degradation

Hydrolysis

Two hydrolysis studies, one photolysis study, one ready biodegradability study (OECD 301C), one surface water simulation study (OECD 309), two water-sediment studies (SETAC Guideline) and three soil studies were available in the CLH Dossier.

Conclusion

In the ready biodegradability test, biodegradation reached only 4 % in 28 days. In degradation studies in water only, in water/sediment systems and in soil, the parent compound is transformed with half-lives from less than one day up to a few days to the major environmental degradant carbendazim. Carbendazim on the other hand, is more persistent with half-lives of 64.8 days, of 15.1-91.6 days and of 22.0-63.2 days in water only, in water/sediment and in soil, respectively. Carbendazim was found as > 80 % in water and water/sediment studies and as > 10 % after only one day. In soil studies, carbendazim was found as max 75.8 %. Additional degradation products were formed but to a lesser extent compared to carbendazim. Based on the available information, thiophanate-methyl is considered as not rapidly degradable for classification purposes.

Bioaccumulation

Experimental data on bioaccumulation (BCFs) are absent. A log K_{ow} value = 1.45 for thiophanate-methyl was calculated following OECD TG 107 Shake Flask Method. For carbendazim, a log K_{ow} = 1.5 was reported. Both values are below the CLP cut-off value of 4. Based on this, the potential for bioaccumulation is considered to be low.

Aquatic hazards

Acute Aquatic Toxicity

The available and reliable data on acute toxicity of thiophanate-methyl, its major environmental degradant carbendazim and additional degradation products to aquatic organisms is summarised in the table below.

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Fish					
Oncorhynchus mykiss	Thiophanate-methyl	Acute 96 hr (flow-through)	Mortality, LC_{50}	11.0 (7.6 – 13) (mm)	Report number 821-001
Ictalurus punctatus	Carbendazim*	96 hr	Mortality, LC_{50}	**0.019** (nom)	Report number A30119; cited in EFSA (2010) *
Oncorhynchus mykiss	Carbendazim*	96 hr	Mortality, LC_{50}	0.19 (nom)	Report number A52914; cited in EFSA (2010) *
Oncorhynchus mykiss	4-OH-TM	96 h, static	Mortality, LC_{50}	>10 (mm, filtrated)	Report number 821-008
Oncorhynchus mykiss	CM-0237	96 h, static	Mortality, LC_{50}	>0.14 (mm, filtrated)	Report number 821-009
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	48 h, flow-through	EC_{50} NOEC	**5.4 (4.4-6.3)** (mm) <4.2 (mm)	Putt, A.E. (1992)
Daphnia magna	BAS 325 10 F	48 h, static	EC_{50} NOEC	4.4 (mm) 3.1 (nom)	Elendt-Schneider (1991)
Daphnia magna	Carbendazim*	48 h	EC_{50}	0.15 (nom)	Fisher (1988), cited in EFSA (2010) *
Daphnia magna	4-OH-TM	48 h, static	EC_{50}	>17.6 (mm, filtrated)	Fujikake, N. (2012)
Daphnia magna	CM-0237	48 h, static	EC_{50}	>0.27 (mm, filtrated)	Fujikake, N. (2012)
Chironomus riparius	4-OH-TM	48 h, static	EC_{50}	>14.6 (mm, filtrated)	Kley, A.. Wydra. V. (2012)
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	E_rC_{50}	37.2 (25.6 – 169) (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	E_rC_{50}	27.3 (22.4 – 33.2) (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	E_rC_{50}	> 11 mg/L (mm)	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	E_rC_{50}	> 15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	E_rC_{50}	> 0.182 (mm)	Baba, K. (2012)
Higher plant					
No data, not needed					

Mm = mean measured

Nom = nominal

The lowest acute endpoint for thiophanate-methyl corresponds to a test with *Daphnia magna* done according to OECD TG 202 with an EC_{50} = 5.4 mg/L. However, due to the rapid conversion of thiophanate-methyl to carbendazim (up to ca 80 % in one week in aquatic test systems), the DS proposes that the toxicity of this degradation product is relevant for the classification.

There are two acute fish studies for carbendazim. In the first study, a non GLP test following the ASTM Guideline (Report A30119), the toxicity to fish (*Oncorhynchus mykiss, Ictalurus punctatus* and *Lepomis macrochirus*) of carbendazim at 10, 22 and 22 °C, respectively, was evaluated. Ten animals per tested concentration were used and an EC_{50} (96h) = 0.019 mg/L was obtained for *I. punctatus* (*channel catfish*) showing that this species is more sensitive to the substance than *O. mykiss* (EC_{50} = 0.87 mg/L) and *L. macrochirus* EC_{50} > 3.2.

In the second fish study (report A52914) 10 *Oncorhynchus mykiss* per tested concentration (control, 0.10, 0.14, 0.24, 0.32, 0.42, 0.56, 0.75, 1 and 2.1 mg/L) were exposed to carbendazim in a static system resulting in an EC_{50} = 0.19 mg/L. The results of this test are considered valid by the DS. The test does not follow any Guideline and is not GLP.

For aquatic invertebrates, Fischer (1988) assessed the toxicity of carbendazim to *Daphnia magna* in a static-acute toxicity test according to OECD TG 202 and following GLP. Four tests (test A, B, C, D) with two different vehicles (HCl and acetone) and with different series of test substance concentrations were performed. Test A had a concentration range from 0.1 to 1 000 mg/L with

HCl as a vehicle. In Test B and C, the concentration ranged from 0.001 to 10 mg/L, using acetone as a vehicle. In Test D, tests b was replicated using HCl as vehicle.

Results showed that in the test systems with Acetone as solvent, the NOEC ranged from 0.032 (test system C) to 0.0056 (test system B). However, in these tests the substance recovery ranged between 16 and 3 247. Results of test system A (HCl as solvent) were regarded as invalid, since the control vessel showed a contamination with the tested substance when analysed, even if no immobilization of the daphnids was observed. Test D was considered a valid result and an EC_{50} = 0.15 mg/L was obtained based on nominal concentrations (concentrations within the HCl system remain within ± 20 of nominal).

For algae a test was done following OECD TG 201 and GLP (Bell, 1996). In the test, nominal concentrations of 0.46, 1.0, 2.2, 4.6 and 10 mg carbendazim/L were applied. Measured concentrations were within ± 20 of nominal and validity criteria were fulfilled according to the DS. The toxicity of carbendazim resulted in an EC_{50} (72 h) > 11 mg/L.

Based on the rapid and considerable generation of carbendazim and that this is more toxic than the parent molecule, thiophanate-methyl, the DS proposes classifying thiophanate-methyl as Aquatic Acute 1; H400 (M = 10) based on the LC_{50} of 0.019 mg/L for carbendazim in *Ictalurus punctatus*.

Chronic Aquatic Toxicity

The available and reliable data on chronic toxicity of thiophanate-methyl, its major environmental degradant carbendazim and additional degradation products to aquatic organisms, is summarised in the table below.

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Laboratory tests					
Fish					
Danio rerio	Thiophanate-methyl	35 days, ELS, flow-through	NOEC EC_{10}	not reported 0.39 (mm)	Report number 826-002 (2014)
Danio rerio	Carbendazim	35 days, ELS, flow-through	NOEC EC_{10}	Not reported	Report number 826-002 (2014)
Oncorhynchus mykiss	Carbendazim*	21 days, flow-through	NOEC	0.0032 (nom)	Report number A40788, cited in EFSA (2010) *

Group	Test substance	Time-scale (Test type)	End point	Toxicity (mg a.s./L)	Reference
Aquatic invertebrates					
Daphnia magna	Thiophanate-methyl	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.16** (mm) Not reported Not reported	Handley, J.W. et al. (1990) ; Wirzinger and Ruhnke (2016)
Daphnia magna	TOPSIN M WDG (Thiophanate-methyl)	21 d, aged test item*	NOEC EC_{10}	0.0373 (mm) 0.0285 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)
Daphnia magna	TOPSIN M WDG (Carbendazim)	21 d, aged test item*	NOEC EC_{10}	0.0177 (mm) 0.0149 (mm)	Schäfers, C. (2007); Schäfers, C. (2016)
Daphnia magna	Carbendazim*	21 d, semi-static	NOEC EC_{10} EC_{20}	**0.0015** (nom) Not reported Not reported	Kelly et al. (1997), cited in EFSA (2010) *
Sediment-dwelling organisms					
Chironomus riparius	Thiophanate-methyl	28 d, water spiked	NOEC EC_{10} EC_{20}	0.44 (init. meas.) Not reported Not reported	Memmert. U. (2002)
Chironomus riparius	Carbendazim*	28 d, water spiked	NOEC EC_{10} EC_{20}	0.0133 (nom) Not reported Not reported	Sowig & Gosch (2002), cited in EFSA (2010) *
Algae					
P. subcapitata	Thiophanate-methyl	72 h, static	NOE_rC	n.d. (mm)	Saito, S. (2002), and Wirzinger, G. (2015)
S. subspicatus	Topsin 500 SC	72 h, static	NOE_rC	3.28 (mm)	Kley, A., Wydra, V. (2012); Wirzinger & Ruhnke (2016)
P. subcapitata	Carbendazim*	72 h, static	NOEC	Not reported	Bell (1996), cited in EFSA (2010) *
P. subcapitata	4-OH-TM	72 h, static	NOEC	15 (mm, dissolved)	Baba, K. (2012)
P. subcapitata	CM-0237	72 h, static	NOEC	0.182 (mm, dissolved)	Baba, K. (2012)

In the Bell (1996) study with *P. subcapitata* a $NOEC_{biomass}$ = 2.5 mg/L was reported.

Mm = mean measured

Nom = nominal

As for the acute toxicity, the DS considers carbendazim data relevant to classify the substance.

There are two studies presented for fish chronic toxicity. The first study corresponds to a chronic test with thiophanate-methyl following OECD TG 210 and GLP (Report 826-002) where the concentrations of carbendazim in the different treatments were also measured. The nominal test concentrations of thiophanate-methyl were 0.125, 0.250, 0.500, 1.000 and 2.000 mg a.s./L. The mean measured test concentrations were between 93.5 % and 103 % of the nominal values. Thus, the treatment levels were defined as 0.123, 0.256, 0.509, 0.939 and 1.870 mg a.s./L. For carbendazim, the mean concentrations per treatment were found to be between 6.3 and 21.0 % of the carbendazim equivalents of the nominal concentrations of thiophanate-methyl (the nominal concentrations of carbendazim were calculated using the molar weight quotient of carbendazim: thiophanate methyl of 0.55838) and were calculated to be 0.0146, 0.0209, 0.0281, 0.0442 and 0.0699 mg carbendazim/L.

The test fulfilled the validity criteria. Results showed an EC_{10} = 0.390 mg a.s./L for thiophanate-methyl. A NOEC/EC_{10} value for carbendazim was not reported, although the DS submitter estimated it to be ca. 0.020 mg/L.

In the second study, the effect of carbendazim to 10 months old *Oncorhynchus mykiss* was assessed in a 21-day prolonged OECD TG 204 test resulting in a NOEC = 0.0032 mg/L.

The chronic toxicity of carbendazim to *Daphnia magna* (Schäfers, 2007, 2016) was investigated testing TOPSIN M WDG (70.5 % thiophanate-methyl) at nominal concentrations of 0.024, 0.043,

0.078, 0.140 and 0.252 mg formulation/L in a test following OECD TG 211. The test duration was 21 days and started at the peak of carbendazim formation in the ageing solution. This occurred around day 8 when carbendazim reached equilibrium between 60 and 70 % of initial thiophanate-methyl loadings. The test fulfilled validity criteria of the updated OECD TG 211 (2012). The overall NOEC for the most sensitive endpoint (mean number of young per female) after 21 days was determined to be 0.078 mg Topsin/L, corresponding to 0.054 mg thiophanate-methyl/L (based on nominal, 0.0373 mg/L based on mean measured concentrations). The corresponding NOEC for carbendazim was 0.0177 mg/L based on mean measured concentrations, the EC_{10} for the same endpoint was calculated to 0.0149 (95 % CL 0.0099 – 0.0189) mg/L. In the test, the DS indicated that the toxicity of thiophanate-methyl is lower than that of carbendazim and proposed that the effects observed in this test are likely due to the presence of carbendazim.

A second study (Kelly *et al*., 1997) measured the toxicity of carbendazim to *Daphnia magna* in a test done according to OECD TG 202. Five concentrations were tested: 0, 0.0018, 0.0056, 0.018, 0.056, 0.18 mg/L. Mean measured concentrations were maintained within ± 20 % nominal (0, 0.0015, 0.0046, 0.015, 0.045, 0.19 mg/L). Results showed a concentration-response curve with a gentle slope regarding the effects on the reproductive performance of *Daphnia magna* in the lower concentration range tested (< 0.015 mg/L). When exposed to the next tested concentration (0.045 mg carbendazim/L), reproduction was reduced by 94 % of control values (5 juveniles/adult). At 0.19 mg/L, the mortality was 100 % and hence no reproduction could be assessed. In this test a NOEC = 0.015 mg/L was obtained.

In addition, a further test studied the chronic toxicity to *Chironomus riparius* of carbendazim according to OECD TG 219. In the test a formulation with 42.6 % of carbendazim was added at concentrations 0, 0.001, 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032 mg/L. Measured concentration levels were between 69.4-74.8 % to nominal concentrations. Since dissipation of the test item from the water phase to the sediment is to be expected in water-sediment systems, the biological results are based on initial nominal figures. The NOEC for *Chironomus riparius* exposed to carbendazim in a 28 day chronic toxicity test was 0.032 mg /L, corresponding to 0.0133 mg carbendazim/L.

In the case of algae (Bell, 1996), the toxicity of carbendazim resulted in a $NOEC_{biomass}$ = 2.5 mg/L.

According to the DS the lowest acute toxicity value for the parent compound thiophanate-methyl was 4.4 mg/L, determined with *Daphnia magna*. However, the lowest acute toxicity value for the major degradant carbendazim was 0.019 mg/L for *Ictalurus punctatus*. As the EC_{50} is between 0.01 and 0.1 mg/L, an M-factor of 10 is proposed.

Based on available data, the DS concludes that thiophanate-methyl and its major degradation product carbendazim are not rapidly degraded in the aquatic environment. The substance has also a low potential for bioaccumulation. According to the DS, the lowest chronic toxicity value for the parent compound thiophanate-methyl was the NOEC = 0.16 mg/L, determined with *Daphnia magna*. However, the lowest chronic toxicity value for the major degradant carbendazim was the NOEC = 0.0015 mg/L, determined with *Daphnia magna*. As the NOEC is between 0.001 and 0.01 mg/L and thiophanate-methyl does not fulfil the criteria for rapid degradation, an M-factor of 10 is proposed.

Based on the rapid and considerable generation of carbendazim and that this is more toxic than the parent molecule, thiophanate-methyl, the DS proposes classifying thiophanate-methyl as Aquatic Chronic 1; H400 (M = 10) based on the $NOEC_{immobility}$ of 0.0015 mg/L for carbendazim in *Daphnia magna*.

Comments received during public consultation

Three Member States (MS) and a Manufacturer commented on the CLH report.

One MS agreed that the substance is not rapidly degradable and the aquatic degradant carbendazim is relevant for hazard classification. However, the MS pinpointed that important data is not available to confirm the validity of various studies of this degradation product. In particular it referred to five studies:

- The acute study with *Ictalurus punctatus*, where it argues that no details are provided for the exposure concentration range, test system media, treatment preparation, control data, validity criteria, etc. This information is relevant to support the reliability of the study and its use as the most sensitive result. It also questioned the relevance of *Ictalurus punctatus* for the test since it is a non-standard species.
    - RAC agrees that the summary provided lacks relevant information which RAC cannot verify. However, the test was performed according to ASTM Guideline and has a reliability of 2. RAC also considers that the species and size used is relevant for acute toxicity. This species is included in USEPA Guidance 850.1075 Fish Acute Toxicity Test, Freshwater and Marine. The study is therefore relevant for classification.

- The acute test with *Oncorhynchus mykiss* (Report A52914), indicating that the study was not conducted according to GLP or a recognised validated test guideline and requesting further information on the reliability of the test, the use of nominal values, recalculation of the endpoint and fulfilment validity criteria.
    - RAC agrees that information to independently assess the reliability, validity of the test, or reasons for recalculation, is missing. The test does not follow any Guideline and is non GLP. The study will be used as supporting information.

- The acute study with *Daphnia magna* (Fischer, 1988), where validity criteria cannot be checked and no justification is provided for the use of nominal values.
    - RAC agrees that the information provided does not allow the possibility to evaluate the validity criteria. However, this study is also included in the carbendazim dossier where the test was given reliability 2 and it can be seen that validity criteria are met. In the control, not more than 10 per cent of the Daphnia should have been immobilised or trapped at the surface of the water. The dissolved oxygen concentration at the end of the test should be 60 per cent of the air saturation value at the temperature used. The use of nominal values is also justified since recovery is within ± 20 % of the nominal concentration when HCl was used. This study is considered valid for classification.

- The chronic study for *O. mykiss* using OECD TG 204 which is not a valid Guideline for chronic toxicity assessment. RAC agrees, this test cannot be considered a suitable long-term test. According to ECHA Guidance on Information Requirements and Chemical Safety Assessment tests performed according to OECD TG 204 (Fish, Prolonged Toxicity Test: 14-Day Study (OECD 1984)) or similar guidelines cannot be considered suitable long-term tests.

- The chronic study for Daphnia (Kelly *et al*., 1997), for which it is unclear if nominal or measured concentrations were used. The MS also asks to check validity against current guidance and for an acute 48-h EC_{50} in support of the Fischer 1988 acute endpoint.
    - Based on the summary provided RAC understands that the endpoint is based on measured concentration. The mortality of the parental generation in the control

was 10 % at the end of the test and therefore met the requirement of the guideline of < 20 %. At the same time the number of live neonates produced in controls was 2 829 in total or 71 per adult fulfilling criteria. A 48-h EC_{50} for this test is not provided, however RAC considers the acute test for daphnia valid. In addition, in this test daphnia are fed, a 48h EC_{50} would not be fully representative of acute toxicity. RAC is of the opinion that this test is valid for classification purposes.

Another MS stressed that studies with preparations of the active substance are not relevant for classification. RAC agrees with the comment and is of the opinion that formulation data can be used when the effect of the active substance is clearly determined. In the CLH Report, the following formulations are used for ecotoxicity tests BAS 32510F, Topsin 500 SC, AE F017411 00 SC42 A208 and Topsin M WDG. For the three first formulations the concentration of the a.s. (thiophanate for the two first and carbendazim for the third) is not higher than 50 %. With such a low concentration, RAC considers the endpoint obtained not relevant for classification. In the case of Topsin M WDG, purity of the a.s. is > 70 %. However, in the test the effect of the active substance remains unclear since it transforms to carbendazim. RAC also considers the endpoints obtained using formulations are not relevant for classification of the active substance as it is not possible to accurately determine the degree to which the active substance products any observed effects.

The third MS agreed with the proposed classification.

In addition a manufacturer disagreed with the proposed classification as Aquatic Acute 1; H400 with M-factor = 10 based on the lowest endpoint for carbendazim (0.019 mg/L for fish). RAC agrees with the DS in using carbendazim data for classification of thiophanate-methyl due to its rapid conversion to carbendazim and its higher toxicity.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS and considers thiophanate-methyl to be not rapidly degradable based on the results of a range of biodegradation studies in various environmental compartments.

Bioaccumulation

With a log K_{ow} = 1.45, below the cut-off value of 4, RAC agrees with the DS and considers the substance non bioaccumulative. For carbendazim a log K_{ow} = 1.5 was reported. Also below the cut-off value of 4.

Acute Aquatic Toxicity

RAC agrees with the DS and considers carbendazim (CAS 10605-21-7) data relevant for classification since thiophanate-methyl rapidly transforms into this degradation product in water, water-sediment and soil and is more toxic than the parent. Thus thiophanate-methyl classification will be based on carbendazim data.

In the thiophanate-methyl dossier there is valid acute data for carbendazim for three trophic levels:

Fish - *Ictalurus punctatus*: LC_{50} (96h) = 0.019 mg/L

Invertebrates - *Daphnia magna*: EC_{50} (48h) = 0.15mg/L

Algae - *Pseudokirchneriella subcapitata* EC_{50} (72h) > 11 mg/L

The lowest endpoint corresponds to *Ictalurus punctatus* LC_{50} = 0.019 mg/L, which is below the cut-off value 1mg/L. In this same study, acute toxicity tests also were conducted with yolk-sac fry, swim-up fry, 0.2 g fry and 1.2 g fingerlings of *O. mykiss* and *I. punctatus*. From these additional endpoints, the most sensitive species was again *I. punctatus* (yolk-sac fry) for which an LC_{50} = 0.007 mg/L nominal was obtained, although RAC concluded that this was not suitable for classification (discussed under In-depth analysis by RAC). Following Table 4.1.0 and Table 4.1.3 of the CLP Regulation, RAC agrees with the DS that thiophanate-methyl warrants classification as Aquatic Acute 1, M = 10 based on the *Ictalurus punctatus* LC_{50} = 0.019 mg/L ($0.01 < L(E)C_{50} \leq 0.1$ mg/L).

For chronic toxicity data is available for two trophic levels:

Invertebrates - *Daphnia magna* NOEC (21d) = 0.0015 mg/L

Algae - *Pseudokirchneriella subcapitata* $NOEC_{biomass}$ (72h) = 2.5 mg/L. RAC acknowledges that growth rate data is preferred to biomass if available.

Chronic toxicity data for fish is not available. RAC considers the test for fish following OECD TG 204 not suitable for the assessment of chronic toxicity under CLP. In the test with *Danio rerio*, the DS estimated an EC_{10} = 0.02 mg/L for carbendazim. Yet this test was done with thiophanate-methyl and therefore RAC is of the opinion that this endpoint is not representative of carbendazim toxicity.

Hence, the lowest endpoint corresponds to the test done with *Daphnia magna* NOEC = 0.0015 mg/L, which falls within the $0.001 < NOEC \leq 0.01$ range. As the substance is not rapidly degradable and following Table 4.1.0(b)(i) and Table 4.1.3 of the CLP Regulation, RAC agrees with the DS that thiophanate-methyl warrants classification as Aquatic Chronic 1, M = 10.

In addition, RAC also assessed the chronic toxicity based on the surrogate approach since there are not adequate chronic data for fish following table 4.1.0(b)(iii) of CLP. Based on the acute most sensitive endpoint for fish, being between $0.01 < L(E)C_{50} \leq 0.1$, classification as Aquatic Chronic 1, M = 10, is warranted.

In summary, thiophanate methyl in not rapidly degradable, has a low potential for bioaccumulation and **warrants classification as Aquatic Acute 1; H400 (M = 10) and Aquatic Chronic 1; H410 (M = 10).**

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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).