Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

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

CLH-O-0000001412-86-281/F

Adopted

15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON THIOPHANATE-METHYL (ISO); DIMETHYL (1,2-PHENYLENEDICARBAMOTHIOYL)BISCARBAMATE; DIMETHYL 4,4'-(O-PHENYLENE)BIS(3-THIOALLOPHANATE)

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance 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
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	1
Comment received				
The German CA agrees with the proposed classification.				
Dossier Submitter's Response				
Noted, thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	Netherlands		MemberState	2
Comment received				
Based on the available information, the NL-CA agrees with the dossier submitter not to classify for physical hazards, skin corrosion/irritation, serious eye damage/irritation, STOT SE and reproductive toxicity. Additionally the NL-CA agrees to retain classification for acute Tox. 4 (inhalation route) and skin sensitization. However, for acute inhalation toxicity it is suggested to propose an ATE value.				
Dossier Submitter's Response				
Agreed. The proposed ATE value is 1.7 mg/L.				
RAC's response				
Agreed.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON THIOPHANATE-METHYL (ISO); DIMETHYL (1,2-PHENYLENEDICARBAMOTHIOYL)BISCARBAMATE; DIMETHYL 4,4'-(O-PHENYLENE)BIS(3-THIOALLOPHANATE)

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	3

Comment received

CLH Report, chapter 11.1 Rapid degradability of organic substances

Page 64: Hydrolysis study on Thiophanate-methyl, Soeda Y. & Nomura O. (1986):
"pH 7: AV-1951 (8.1% after 33 days), half-life not available"
AND
"pH 9: AV-1951 (24.9% after 4 days). DT50 5.4 days (SFO-SFO) but this DT50 was considered uncertain."

At pH9 and 22°C a decline of the metabolite was observed at the end of the study, so an appropriate kinetic assessment was possible. The calculations resulted in the DT50 value of 5.4 h using SFO kinetics had a Chi² error of <1.0 % and a p-level of the t-test of <2E-16. Furthermore the visual assessment was good. As the residue level at the end of the test used in the kinetic assessment was close to the maximum occurrence, the DT50 value of 5.4 h for AV-1951 can be considered as conservative.

Page 64: Study on degradation of Thiophanate-methyl in lake water (pelagic system) at 20°C

It should be noted that metabolites UM-2 and 2-AB occurred only occasionally. In general the main purpose of this study type is to address mineralization. The degradation rate of the metabolites in the aerobic mineralisation study is not part of the data requirements and are often uncertain due to the low test concentration.

Page 64: Water/sediment study on Thiophanate-methyl, in pond and river systems, at 20°C, Voelkl S. (2001)
"Observed metabolites in pond system: […] AV-1951, max 6.1% (2 days), half-life not available" and "Observed metabolites in river system: […] AV-1951, max 6.3% (2 days), half-life not available"

It should be noted that AV-1951 is not a major metabolite of Thiophanate-methyl according to the data requirements of plant protection actives.

CLH Report, chapter 11.5.1 Acute (short-term) toxicity to fish

Page 137: Authors of study should be blackened as it is a vertebrate study

Page 140: Authors of study should be blackened as it is a vertebrate study

CLH Report, chapter 11.6 Long-term aquatic hazard

Page 173 (Table 160): The applicant does not agree that the endpoint for the chronic Chironomus study with Thiophanate-methyl is based on the initial measured concentrations as they were within ± 20 % of the nominal concentrations. In accordance with the OECD guideline, if this is the case, then the endpoints may be based on nominal concentrations.

CLH Report, chapter 11.6.2 Chronic toxicity to aquatic invertebrates

Page 198: The applicant does not agree that the endpoint for the chronic Chironomus study with Thiophanate-methyl is based on the initial measured concentrations as they were within ± 20 % of the nominal concentrations. In accordance with the OECD guideline, if this is the case, then the endpoints may be based on nominal concentrations.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Carcinogenicity comments SCC_1.zip

Dossier Submitter's Response

CLH Report, chapter 11.1: Page 64: Hydrolysis study on Thiophanate-methyl, Soeda Y. & Nomura O. (1986)
Please note that the estimated DT50 for AV-1951 at pH 9 (22º) was 5.4 days, not hours. The RMS has accepted the DT50 of 5.4 days as the best available estimate of the hydrolytic stability of AV-1951. The result was considered as uncertain - mainly due to the fact that the the DT50 was extrapolated beyond the duration of the study. The fact that parameters estimated simultaneously for carbendazim (MBC) were unreliable also added to the uncertainty. Please see under "RMS comments and conclusion" for this particular study in section 11.1.3. Whether or not the estimated DT50 5.4 days for AV-1951 can be considered as conservative would not seem to be of importance in relation to the proposed classification.

CLH Report, chapter 11.1: Page 64: Study on degradation of Thiophanate-methyl in lake water (pelagic system) at 20°C(Hurst, 2015)
In both experiments (low and high dose) UM-2 was only detected at the final samling point (day 30) and 2-AB was only observed at the two final sampling points (days 14 and 30), and the highest amounts were observed at the very last sampling point (see Table 73 in section 11.1.4.3). It is agreed that the main purpose of the study (OECD TG No 309) is to investigate mineralisation. The results were accepted despite the fact that DT50s could not be estimated for all metabolites.

CLH Report, chapter 11.1: Page 64: Water/sediment study on Thiophanate-methyl, in pond and river systems, at 20°C, Voelkl S. (2001)
Agree that since metabolite AV-1951 was only observed as >5% of the applied radioactivity at one single sampling point in each test system the metabolite was not included in the definition of residues requiring further assessment, as defined under Regulation (EC) No 1107/2009. See Tables 80 and 81 in section 11.1.4.3.

CLH Report, chapter 11.5
Agree that the authors of all vertebrate studies should have been blackened.

CLH Report, chapter 11.6
Not agreed. From our opinion, measured test concentrations is preferred when available. In this case (Memmert, 2002), the initial measured was 88% of the nominal value.

RAC's response

CLH Report, chapter 11.1: Page 64: Hydrolysis study on Thiophanate-methyl, Soeda Y. & Nomura O. (1986).
RACs agrees with the DS interpretation considering X^2, p-values and duration of the study. RAC is also of the thought that whether or not the estimated DT50 5.4 days for AV-1951 is conservative is not relevant in relation to the proposed classification.

Page 64: Study on degradation of Thiophanate-methyl in lake water (pelagic system) at 20 °C. RAC agrees with the DS response.

CLH Report, chapter 11.1: Page 64: Water/sediment study on Thiophanate-methyl, in pond and river systems, at 20 °C, Voelkl S. (2001).

CLP Guidance indicates that "when a substance so tested may degrade to give rise to a more hazardous product…the classification of the parent compound should take due account of the hazard of the degradation product, and the rate at which it can be formed under normal environmental conditions." RAC considers these are the aspects to look at when assessing a metabolite and not only the percentage formed.

CLH Report, chapter 11.5. Noted

CLH Report, chapter 11.6. There is no reference in Guidaline OECD 219 in relation to the concentration to be used when test substance concentration is maintained within ±20 nominal. Guidance OECD 211 on chronic toxicity to invertebrates indicates *"If there is evidence that the concentration of the substance being tested has been satisfactorily maintained within ± 20 per cent of the nominal or measured initial concentration throughout the test, then results can be based on nominal or measured initial values."* Thus an endpoint based on measured initial values is also valid.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	4
Comment received				
1.1. Table 1, page 5: - According to the List of Endpoints of the RAR (November 2017), the IUPAC name of the substance thiophanate-methyl is dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate) and the CA name is dimethyl N,N'-[1,2-phenylenebis(iminocarbonothioyl)]bis[carbamate]. - Topsin M is the name of the representative formulation of the RAR, it should not be proposed as another name for thiophanate-methyl. Table 2.1., page 12: The DS proposal for classification carc.2 H351 (refer to page 40), is not reported in the table 2.1. Could you please clarify?				
Dossier Submitter's Response				
We agree that the IUPAC-name and CA-names are not consistent with those used in the RAR. The names were changed in the CLH-dossier upon ECHA's request in the accordance check. It seems that the change of the CA-name should only be done if it has changed in the CAS-registry and we are not aware that this has been done. With regards to the other name for thiopahanate-methyl, Topsin M this is actually a synonym (see for example PubChem). The representative formulation is denoted Topsin M 500 SC. Table 2.1., page 12: The omission of the DS proposal for classification carc.2 H351 is table 2.1 was a mistake. However, please refer to comment 8.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	5
Comment received				
Please correct Table 6 to include Carc. 2 proposal. According to chapter 10.9.3, the DS clearly proposes classification as Carc. 2, H351. This would be also in agreement with the pesticide peer review process (EFSA 2018, doi: 10.2903/j.efsa.2018.5133, page 10).				
Dossier Submitter's Response				
Please refer to comment 8.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	Netherlands		MemberState	6
Comment received				
Carcinogenicity Page no. 34 - 40 The carcinogenicity in line with the CLP criteria is a borderline case. The criteria for carc. 2 seem to be met on the basis of benign neoplasms or lesions seen in a dose-dependent manner in two species with different tumor types. Further, The NL-CA notes that the substance has a structural alert when put through the OECD toolbox (thiocarbonyl) for non-genotoxic carcinogenicity. Since this is a borderline case, it may be helpful to include this QSAR information as additional support. There is a publicly available EPA report with information on the carcinogenicity of thiocarbonyl compounds. In this report, other thiocarbonyl compounds are mentioned as examples but most importantly, the type of carcinogenicity observed is similar (predominantly thyroid hypertrophy/hyperplasia by inhibiting thyroid hormones resulting in tumors, but also liver tumors in mice as also observed for thiophanate-methyl). Therefore this similarity can be used as support for likely carcinogenicity. The observations in favor of classification: 1. The studies summarized in the CLH proposal indicate significant carcinogenic effects at high doses. 2. Less pronounced, predominantly non-significant incidences were also observed after lower exposures to thiophanate-methyl in the absence of excessive toxicity, indicating a dose-response relationship. 3. Proliferation was observed in the tumour forming organs before tumour formation and also at lower dose levels. Observations unsupportive for classification: 1. The significant carcinogenic effects occurred in the presence of mortality in the key study with mice, which may be partially attributable to the tumors. Other adverse effects were seen as well in the key rat study together with the thyroid tumors (which may be interrelated), such as nephropathy (although not in the female rats). 2. The tumor types are predominantly benign. 3. Additional information suggests that the tumors may be caused by a mechanism that is not relevant for humans (phenobarbital like), although this has not been demonstrated sufficiently. In addition, the EPA report on thiocarbonyl compounds suggests otherwise, while the phenobarbital like mode of action applies mostly to the liver tumors, this does not apply to the thyroid tumors.				

Overall, there is a dose-dependent increase in the tumor incidence of benign tumors. The NL-CA is inclined to agree with the proposal for Carc. 2, also considering the support from thiocarbonyl compounds that predominantly induce the same tumor types as observed for thiophanate-methyl (see EPA report for a start). We therefore suggest to use this information to further support the proposal for Carc. 2. Reference: David Y. Lai, Yin-tak Woo, Joseph C. Arcos, Mary F. Argus, Thiocarbonyl Compounds: Carcinogenicity and Structure Activity Relationships (1982)
Dossier Submitter's Response
Please refer to comment 8.
RAC's response
See response to comment 9.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	7
Comment received				
The proposal for classification Carc. 2 H351 is agreed upon.				
Dossier Submitter's Response				
Please refer to comment 8.				
RAC's response				
See response to comment 9.				

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	France	SCC Scientific Consulting Company GmbH	Industry or trade association	8
Comment received				
Reference: CLH Report section 10.9.3 (page 40) Comment by Prof. Dr. Gelbke The DS stated 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 met in the rat study but not in the mouse study. Overall, data is considered as "limited" evidence of carcinogenicity and classification in Carc. 2 H351 is proposed for thiophanate-methyl." In conclusion, the liver tumors in mice as well as the thyroid tumors in rats are of no relevance for hazard identification in humans. Therefore a classification of TM for carcinogenicity is not appropriate. Additional relevant publications are provided herewith. For details please refer to Gelbke (2018a). The following attachments are provided with this comment:				

1) Battershill, JM, Fielder, RJ (1998). Mouse-specific carcinogens: an assessment of hazard and significance for validation of short-term carcinogenicity bioassays in transgenic mice. Hum. Exp. Toxicol. 17: 193-205
("Battershill and Fiedler _1998.pdf")

2) Haseman, JK, Hailey, JR, Morris, RW (1998). Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F, Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update ("Haseman_1998.pdf")

3)RIVM report 601516009/2002 Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment (RIVM), Part II Editors: R. Luttik and S.M.G.J. Pelgrom ("RIVM report.pdf")

4) Gelbke, HP (2018a). Thiophanate methyl (TM): classification for carcinogenicity ("Gelbke_2018a carcinogenicity of Thiophanate-methyl.pdf")

Dossier Submitter's Response

In the first CLH dossier for TM submitted by the DS, no classification for carcinogenicity was proposed for TM. This was changed after the EFSA Peer Review to reflect the outcome of the EFSA Expert meeting for TM which concluded that: "t*he rat carcinogenesis showed a dose related increase in follicular cell adenoma/carcinoma in male rats with a trend of follicular cell adenoma in female rats. There was an increase in mortality in the top dose group which was partially linked to the thyroid tumours. Although the high rate of mortality could, at least in part, be due to excessive toxicity, the treatment relationship of thyroid tumours was considered real. The experts considered the carcinogenic effects observed in the mouse and rat as enough evidence to support classification as Carc. Cat. 2."*

However, the DS agrees with Gelbke, HP (2018a) that the MTD was problably exceeded at the highest dose level in the 2-year rat study where the statistically significant increase in adenomas were noted. At the dose below, adenoma was noted in 4/60 males and 1/60 females (not statistically significant). No HCD are available. Contrary to the opinion of the EFSA Expert meeting, the DS agrees that the results of this study does not justify classification for carcinogenicity.

The DS disagrees with the argumentation regarding relevance to humans presented in Gelbke, HP (2018a). The only thyroid finding in dogs is said to be increased weight in the 1-year study. However, as stated in the CLH report, there was also a reduction in T4 levels in males and minimal to moderate hypertrophy and slight hyperplasia were noted. In the 90-day study, T3 levels were reduced in females at both 200 and 400 mg/kg bw/day. In spite of marked decreases in terminal body weights, absolute liver (6-11%) and thyroid weights (11-49%) were increased compared to concurrent controls. Statistical significance was however reached for relative weights only. Hypertrophy of the follicular epithelial cells of the thyroid was seen in each treated group, but not in the control group. Minimal to marked hyperplasia of the follicular epithelium was also seen starting from 200 mg/kg bw/day. The fact that effects were seen not only in rats but also in dogs increases the likelihood of relevance to humans.

Regarding the tumours in mice, Gelbke, HP (2018a), also argues that there is a lack of human relevance. For the DS comments on this, please refer to comment 9.

In the mouse carcinogenicity study, hepatocellular adenomas were statistically significantly increased at 3000 and 7000 ppm. In the high dose group, there was a

concomitant statistically significant increased mortality rate. The mortality rate was increased also in the second highest treatment group but this was not statistically significant. The main cause of death was amyloidosis. The DS considers it unclear whether it was caused by the test substance. This effect can arise due to genetic factors, however as the mortality was statistically significant it was apparently more common in the highest dose. In all, it is not considered safe to conclude that the MTD was reached in this study.

The significance of tumours observed in the study is discussed below based on considerations included in the CLP guidance:

(a) tumour type and background incidence;

Table 1: Incidence of mouse hepatocellular adenomas.

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

**p<0.01

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

(b) multi-site responses;
No, hepatocellular adenoma only.

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

(d) reduced tumour latency;
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 second highest treatment group. Hepatocellular adenomas were only observed at terminal sacrifice indicating slow tumour development.

(e) whether responses are in single or both sexes;
The tumours were observed in both sexes.

(f) whether responses are in a single species or several species;
Hepatocellular adenomas were observed mice only.

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
No information available.

(h) routes of exposure;

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

(i) comparison of ADME between test animals and humans;
A comparative in vitro metabolism study is available (RAR Vol 3, B.6.1.4) and showed no differences in metabolism between humans and rats. No such study is available with mice.

(j) the possibility of a confounding effect of excessive toxicity at test doses;
The MTD is not considered to have been reached (see above).

(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 mice (thyroid tumours were observed in rats above the MTD) there are no clear indications of a link between genetic instability and cancer.

Lack of human relevance is not considered to have been shown (see comment 9).

In conclusion, hepatocellular adenomas were observed in both sexes of one species at a dose which may possibly have been above the MTD. Although there are weak indications of a carcinogenetic effect, the DS does not consider that they are severe enough to justify classification for carcinogenicity.

RAC's response

See response to comment 9.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	9

Comment received

Reference: CLH Report section 10.9.3 (page 40)

Applicant's comment (5 of 5):

With regard to the conclusion of the DS (KEMI)on the carcinogenicity of Thiophanate-methyl (TM), several new findings should be considered by the RAC members.

Thiophanate-methyl is not subject to classification with Carc. 2, since the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are secondary to liver enzyme induction and not relevant to humans. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018a).

The following attachments are provided with this comment (submitted in 5 different batches, batch 5 of 5):

1) New studies on thyroid peroxidase (TPO) inhibition demonstrating
a lack of relevant inhibition for human TPO in vitro when tested
up to precipitating concentrations.
- TPO in vitro inhibition in human
(„Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf")

- TPO in vitro inhibition in dog
(„Haines_2018_Dog_TPO _NIP_593-002_RD-10598.pdf")
- TPO in vitro inhibition in pig
(„Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf")
- TPO in vitro inhibition in rat
(„Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf")

2) OECD Tier 2 summaries for TPO inhibition assays submitted
with this comment:
- OECD Tier 2 summaries
(„New studies Thiophanate-methyl TPO ASSAY - tier 2 summaries.pdf")

3) Becker RA, Patlewicz G, Simon TW, Rowlands JC, Budinsky RA (2015). The adverse outcome pathway for rodent liver tumor promotion by sustained activation of the aryl hydrocarbon receptor. Regul Toxicol Pharmacol. 2015 Oct;73(1):172-90. doi: 10.1016/j.yrtph.2015.06.015. (Becker_2015.pdf)

4) Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A (2018). Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 92:1-7. doi: 10.1016/j.yrtph.2017.11.003. (Felter _2018.pdf)

5) Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, Lake BG (2018). Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured mouse, rat and human hepatocytes. Toxicology 396-397:23-32. (Haines _2018.pdf)

6) Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, Wolf CR, Elcombe CR (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol Sci. 116:452-466. (Ross _2010.pdf)

7) Shah I, Houck K, Judson RS, Kavlock RJ, Martin MT, Reif DM, Wambaugh J, Dix DJ (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS ONE 6(2): e14584.
doi:10.1371/journal.pone.0014584 (Shah _2011.pdf)

8) Briese, B.H., Harder V. & Heidemann, A. (2018a): Comments on the carcinogenicity of Thiophanate-methyl (Briese et al_2018a.pdf)

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf

Dossier Submitter's Response

For the DS's view on the carcinogenicity potential of TM, please refer to comment 8.

Regarding the relevance for humans the DS would like to make the following comments:

The effects on thyroid seen in the in vivo studies available include weight increase, hypertrophy, hyperplasia, tumours and effects on thyroid hormones in rats and weight

increase, hypertrophy, hyperplasia and effects on thyroid hormones in dogs. We consider these effects are relevant for human health, regardless if the effects are due to a direct action on the thyroid, or indirectly via liver induction or other mechanisms.

The mechanisms of action are not known but it was suggested in the CLH dossier that it may involve upregulation of UDPGT as well as TPO inhibition. This was shown in a mechanistic study presented in the CLH dossier where, among other findings, TPO inhibition was observed in pig. Nippon Soda Co. Ltd. has submitted four new studies on TPO inhibition in vitro. These show a slight inhibition of TPO in rats and dogs but no such effect in pigs and humans. The results are thereby not consistent with the existing study and no explanation has been provided by Nippon Soda Co. Ltd. There are no guidelines for in vitro studies on thyroid. The DS therefore does not consider that sufficient evidence has been provided that TPO inhibition does not occur. Moreover, there are other possible MoAs that have not been investigated, e.g. inhibition of the sodium-iodide symporter (NIS). In conclusion, the DS does not consider the data submitted to be evidence of lack of human relevance for the effects noted in the in vivo studies in rats and dogs.

Nippon Soda Co. Ltd. has also submitted data showing the likely involvement of four different nuclear receptors (NR)– CAR, PPRAα, PXR and AhR - in the formation of liver tumours in mice. By referring to several cited references they argue that this MoA lacks human relevance. However, the publications discuss human relevance of each NR separately. No data has been submitted to show lack of human relevance for the combination of all these NRs. Simultaneous activation of nuclear receptors is likely to have a distinct biological outcome compared to the separate activiation of the same pathways (please refer e.g. to EFSA Conclusion in propyzamide, 2016) and therefore the DS does not consider that lack of human relevance has been demonstrated.

RAC's response

We agree with the DS statements that: i) "The effects on thyroid seen in the *in vivo* studies available include weight increase, hypertrophy, hyperplasia, tumours and effects on thyroid hormones in rats and weight increase, hypertrophy, hyperplasia and effects on thyroid hormones in dogs. We consider these effects are relevant for human health, regardless if the effects are due to a direct action on the thyroid, or indirectly via liver induction or other mechanisms"; and ii) "There are no guidelines for *in vitro* studies on thyroid."
Despite this, the provided data on the absence of TPO inhibition upon thiophanate methyl exposure *in vitro* appear quite convincing, although it cannot absolutely be excluded that other mechanistic events could be responsible for both the altered levels of thyroid hormones and for the occurrence of the main histopathological adverse outcome (i.e. hypertrophy and hyperplasia).
As suggested by the DS, the investigation of potential interference on the iodine uptake looking at the activity of the NIS deserve further attention.
Overall, also excluding the TPO inhibition, the induction of an adverse outcome in the thyroid is undeniable and its relevance for human health cannot be excluded.

Regarding the possible involvement of nuclear receptors CAR, PPRAα, PXR and AhR in the etiology of tumours induced in mice by thiophanate-methyl , we agree 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.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	10

Comment received

Reference: CLH Report section 10.9.3 (page 40)

Applicant's comment (4 of 5):

With regard to the conclusion of the DS (KEMI)on the carcinogenicity of Thiophanate-methyl (TM), several new findings should be considered by the RAC members.

Thiophanate-methyl is not subject to classification with Carc. 2, since the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are secondary to liver enzyme induction and not relevant to humans. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018a).

The following attachments are provided with this comment (submitted in 5 different batches, batch 4 of 5):

1) New studies on thyroid peroxidase (TPO) inhibition demonstrating
a lack of relevant inhibition for human TPO in vitro when tested
up to precipitating concentrations.
- TPO in vitro inhibition in human
(„Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf")
- TPO in vitro inhibition in dog
(„Haines_2018_Dog_TPO _NIP_593-002_RD-10598.pdf")
- TPO in vitro inhibition in pig
(„Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf")
- TPO in vitro inhibition in rat
(„Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf")

2) OECD Tier 2 summaries for TPO inhibition assays submitted
with this comment:
- OECD Tier 2 summaries
(„New studies Thiophanate-methyl TPO ASSAY - tier 2 summaries.pdf")

3) Becker RA, Patlewicz G, Simon TW, Rowlands JC, Budinsky RA (2015). The adverse outcome pathway for rodent liver tumor promotion by sustained activation of the aryl hydrocarbon receptor. Regul Toxicol Pharmacol. 2015 Oct;73(1):172-90. doi: 10.1016/j.yrtph.2015.06.015. (Becker_2015.pdf)

4) Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A (2018). Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 92:1-7. doi: 10.1016/j.yrtph.2017.11.003. (Felter _2018.pdf)

5) Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, Lake BG (2018). Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured

mouse, rat and human hepatocytes. Toxicology 396-397:23-32. (Haines _2018.pdf)

6) Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, Wolf CR, Elcombe CR (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol Sci. 116:452-466. (Ross _2010.pdf)

7) Shah I, Houck K, Judson RS, Kavlock RJ, Martin MT, Reif DM, Wambaugh J, Dix DJ (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS ONE 6(2): e14584.
doi:10.1371/journal.pone.0014584 (Shah _2011.pdf)

8) Briese, B.H., Harder V. & Heidemann, A. (2018a): Comments on the carcinogenicity of Thiophanate-methyl (Briese et al_2018a.pdf)

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf

Dossier Submitter's Response
Please refer to comment 9.
RAC's response
See above response to comment 9.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	11

Comment received

Reference: CLH Report section 10.9.3 (page 40)

Applicant's comment (3 of 5):

With regard to the conclusion of the DS (KEMI)on the carcinogenicity of Thiophanate-methyl (TM), several new findings should be considered by the RAC members.

Thiophanate-methyl is not subject to classification with Carc. 2, since the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are secondary to liver enzyme induction and not relevant to humans. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018a).

The following attachments are provided with this comment (submitted in 5 different batches, batch 3 of 5):

1) New studies on thyroid peroxidase (TPO) inhibition demonstrating
a lack of relevant inhibition for human TPO in vitro when tested
up to precipitating concentrations.
- TPO in vitro inhibition in human
(„Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf")
- TPO in vitro inhibition in dog

(„Haines_2018_Dog_TPO _NIP_593-002_RD-10598.pdf")
- TPO in vitro inhibition in pig
(„Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf")
- TPO in vitro inhibition in rat
(„Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf")

2) OECD Tier 2 summaries for TPO inhibition assays submitted
with this comment:
- OECD Tier 2 summaries
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3) Becker RA, Patlewicz G, Simon TW, Rowlands JC, Budinsky RA (2015). The adverse outcome pathway for rodent liver tumor promotion by sustained activation of the aryl hydrocarbon receptor. Regul Toxicol Pharmacol. 2015 Oct;73(1):172-90. doi: 10.1016/j.yrtph.2015.06.015. (Becker_2015.pdf)

4) Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A (2018). Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 92:1-7. doi: 10.1016/j.yrtph.2017.11.003. (Felter _2018.pdf)

5) Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, Lake BG (2018). Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured mouse, rat and human hepatocytes. Toxicology 396-397:23-32. (Haines _2018.pdf)

6) Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, Wolf CR, Elcombe CR (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol Sci. 116:452-466. (Ross _2010.pdf)

7) Shah I, Houck K, Judson RS, Kavlock RJ, Martin MT, Reif DM, Wambaugh J, Dix DJ (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS ONE 6(2): e14584.
doi:10.1371/journal.pone.0014584 (Shah _2011.pdf)

8) Briese, B.H., Harder V. & Heidemann, A. (2018a): Comments on the carcinogenicity of Thiophanate-methyl (Briese et al_2018a.pdf)

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf

Dossier Submitter's Response

Please refer to comment 9.

RAC's response

See above response to comment 9.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	12

Comment received

Reference: CLH Report section 10.9.3 (page 40)

Applicant's comment (2 of 5):

With regard to the conclusion of the DS (KEMI)on the carcinogenicity of Thiophanate-methyl (TM), several new findings should be considered by the RAC members.

Thiophanate-methyl is not subject to classification with Carc. 2, since the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are secondary to liver enzyme induction and not relevant to humans. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018a).

The following attachments are provided with this comment (submitted in 5 different batches; 2 of 5):

1) New studies on thyroid peroxidase (TPO) inhibition demonstrating
a lack of relevant inhibition for human TPO in vitro when tested
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- TPO in vitro inhibition in dog
(„Haines_2018_Dog_TPO _NIP_593-002_RD-10598.pdf")
- TPO in vitro inhibition in pig
(„Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf")
- TPO in vitro inhibition in rat
(„Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf")

2) OECD Tier 2 summaries for TPO inhibition assays submitted
with this comment:
- OECD Tier 2 summaries
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3) Becker RA, Patlewicz G, Simon TW, Rowlands JC, Budinsky RA (2015). The adverse outcome pathway for rodent liver tumor promotion by sustained activation of the aryl hydrocarbon receptor. Regul Toxicol Pharmacol. 2015 Oct;73(1):172-90. doi: 10.1016/j.yrtph.2015.06.015. (Becker_2015.pdf)

4) Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A (2018). Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 92:1-7. doi: 10.1016/j.yrtph.2017.11.003. (Felter _2018.pdf)

5) Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, Lake BG (2018). Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured

mouse, rat and human hepatocytes. Toxicology 396-397:23-32. (Haines _2018.pdf)

6) Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, Wolf CR, Elcombe CR (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol Sci. 116:452-466. (Ross _2010.pdf)

7) Shah I, Houck K, Judson RS, Kavlock RJ, Martin MT, Reif DM, Wambaugh J, Dix DJ (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS ONE 6(2): e14584.
doi:10.1371/journal.pone.0014584 (Shah _2011.pdf)

8) Briese, B.H., Harder V. & Heidemann, A. (2018a): Comments on the carcinogenicity of Thiophanate-methyl (Briese et al_2018a.pdf)

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Carcinogenicity comment SCC_2.zip

Dossier Submitter's Response
Please refer to comment 9.
RAC's response
See above response to comment 9.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	13

Comment received

Reference: CLH Report section 10.9.3 (page 40)

Applicant's comment:

With regard to the conclusion of the DS (KEMI)on the carcinogenicity of Thiophanate-methyl (TM), several new findings should be considered by the RAC members.

Thiophanate-methyl is not subject to classification with Carc. 2, since the tumours noted in rats (thyroid follicular cell adenoma) or mice (hepatocellular adenoma) are secondary to liver enzyme induction and not relevant to humans. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018a).

The following attachments are provided with this comment (submitted in 5 different batches):

1) New studies on thyroid peroxidase (TPO) inhibition demonstrating
a lack of relevant inhibition for human TPO in vitro when tested
up to precipitating concentrations.
- TPO in vitro inhibition in human
(„Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf")
- TPO in vitro inhibition in dog

(„Haines_2018_Dog_TPO _NIP_593-002_RD-10598.pdf")
- TPO in vitro inhibition in pig
(„Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf")
- TPO in vitro inhibition in rat
(„Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf")

2) OECD Tier 2 summaries for TPO inhibition assays submitted
with this comment:
- OECD Tier 2 summaries
(„New studies Thiophanate-methyl TPO ASSAY - tier 2 summaries.pdf")

3) Becker RA, Patlewicz G, Simon TW, Rowlands JC, Budinsky RA (2015). The adverse outcome pathway for rodent liver tumor promotion by sustained activation of the aryl hydrocarbon receptor. Regul Toxicol Pharmacol. 2015 Oct;73(1):172-90. doi: 10.1016/j.yrtph.2015.06.015. (Becker_2015.pdf)

4) Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, Goodman J, Haber LT, Jacobs A, Klaunig JE, Lynch AM, Moggs J, Pandiri A (2018). Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regul Toxicol Pharmacol. 92:1-7. doi: 10.1016/j.yrtph.2017.11.003. (Felter _2018.pdf)

5) Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, Lake BG (2018). Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured mouse, rat and human hepatocytes. Toxicology 396-397:23-32. (Haines _2018.pdf)

6) Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, Wolf CR, Elcombe CR (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol Sci. 116:452-466. (Ross _2010.pdf)

7) Shah I, Houck K, Judson RS, Kavlock RJ, Martin MT, Reif DM, Wambaugh J, Dix DJ (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS ONE 6(2): e14584.
doi:10.1371/journal.pone.0014584 (Shah _2011.pdf)

8) Briese, B.H., Harder V. & Heidemann, A. (2018a): Comments on the carcinogenicity of Thiophanate-methyl (Briese et al_2018a.pdf)

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Carcinogenicity comments SCC_1.zip

Dossier Submitter's Response
Please refer to comment 9.
RAC's response
See above response to comment 9.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	Netherlands		MemberState	14
Comment received				

Mutagenicity
Page no. 27 - 34
Although the NL-CA in general agrees with the discussion and arguments presented by the dossier submitter, the proposed classification for Muta. 1b. is considered questionable.

In support of mutagenicity in somatic cells, the NL-CA has the following considerations in addition to the presented discussion and arguments by the dossier submitter:
1. The in vitro and in vivo positive results were all micronucleus assays. The results of micronucleus assays do not necessarily have to correlate with positive chromosome aberration tests (other than micronucleus tests) or other mutagenic effects (Parry et al., 2002) and therefore the negative results from genotoxicity assays with different endpoints cannot overrule these results.
2. All (considered reliable by the dossier submitter) micronucleus tests (both in vitro and in vivo) are positive except for one, forming clear evidence for mutagenic potential. The single negative result from a study without confirmation of bone marrow exposure and using a different mouse strain should not overrule a seemingly more reliable in vivo assay with a positive result, supported by the in vivo study with lizards and two positive in vitro studies (that used human cells as additional support for human relevance).
3. The reevaluation of the key in vivo mice study with centromeric staining seems to indicate a predominant clastogenic effect. Notably, aneugenic effects can result in carcinogenicity as well (Parry et al., 2002) and should be regarded as relevant even though clastogenic effects may generally be considered as more concerning because of the likely non-threshold mechanism of action. The NL-CA also observes that general chromosome aberration tests may not pick up chemicals with a predominant clastogenic mechanism of action, which could partly explain the positive micronuclei tests opposing the other negative genotoxicity/chromosome aberration studies. However there is some conflicting information since one in vitro micronucleus assay indicated an aneugenic mechanism (but did not exclude a clastogenic one either?) and other tests such as the dominant lethal assays would be expected to pick up clastogenic effects most of the time (but perhaps not always).
4. The NL-CA agrees with the considerations that the results in the "first" in vivo micronucleus test can be regarded as positive although they may fall within the negative control range since there seems to be a dose-response relationship and there is a clear difference with the negative control.

However, when strictly following the CLP guideline, the following considerations lead to the opinion that classification is not warranted for mutagenicity 1b:
The main issue: there is no direct evidence indicating similar mutagenic effects in germ cells. On the other hand, as pointed out by the dossier submitter, the registrant failed to provide firm evidence germ cell mutagenicity does not occur after exposure to thiophanate-methyl. The studies provided testing mutagenicity in germ cells all lacked confirmation of target exposure and had additional deficiencies (e.g. non-GLP or non-guideline, sensitivity differences). Further, the toxicokinetic studies indicate exposure of the gonads to thiophanate-methyl and/or its metabolites. This does not necessarily indicate an interaction between thiophanate-methyl and the DNA of the germ-cells. Although germ cell mutagenicity is plausible, the possible interaction and reaction with

the germ-cell DNA is insufficiently demonstrated (while this demonstration is required according to the CLP guideline).
"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"
The evidence that germ cells are exposed is not considered sufficient indication for a possible interaction between the chemical and the genetic material of germ cells. One explanation for negative results in in vivo germ cells is that the parent, rather than the metabolites is likely responsible for the observed effects since micronucleus tests in vitro were only positive in the absence of S9. This may result in insufficient quantity of the parent to reach the gonads while perhaps more reaches the bone marrow. The kinetic studies utilized a radioactive label to demonstrate the distribution which often cannot discriminate between the parent and metabolite in tissues unless combined with additional analyses. Please specify if available.
Therefore the NL-CA is of the opinion that in accordance with the CLP regulation (criteria), classification as muta. 1b is not warranted for thiophanate-methyl.
Regardless, the NL-CA would like to express sympathy for the proposed classification because the CLP criteria may be regarded as too limited/strict. The clastogenic mechanism of action with clear mutagenic effects in somatic cells with limited support of likely germ cell exposure may be sufficient for identification of a substance as a germ cell mutagen (while that is currently not sufficient for classification in category 1b according to the CLP criteria).

Question: carbendazim is identified as one of the metabolites of thiophanate-methyl and has a harmonised classification as Muta 1b. Did you consider classification based on read-across due to the formation of a metabolite classified as Muta 1b comparable to the formaldehyde releasers? Is there sufficient evidence of the percentage carbendazim formed and do these two substances show a comparable toxicity and mutagenicity profile?

References:

Parry, E. M., Parry, J. M., Corso, C., Doherty, A., Haddad, F., Hermine, T. F., . . . Williamson, J. (2002). Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis, 17(6), 509-521. doi:10.1093/mutage/17.6.509

Dossier Submitter's Response
Please refer to comment 17.
RAC's response
Please refer to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	15
Comment received				
Page 32: It is agreed that the new studies performed with Crl:CD-1 (ICR) mice (spermatogonial chromosomal abberation tests and micronucleus tests in germ cells) are of low reliability and do not rule out the positive results observed in vivo in somatic cell with B6D2F1 mice.				

Such studies would have been more informative if performed with the most sensitive strain (i.e.: B6D2F1 mice). Page 34, point 10.8.3: The proposal for classification Muta. 1B H340 is supported based on the clastogenic and aneugenic potential of thiophanate-methyl in vivo in somatic cells as well as its bioavailability including gonads exposure. Furthermore carbendazim (classified Muta. 1B H340) is a major metabolite of thiophanate-methyl in mammals.
Dossier Submitter's Response
Please refer to comment 17.
RAC's response
Please refer to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	France	SCC Scientific Consulting Company GmbH	Industry or trade association	16

Comment received

Reference: CLH Report section 10.8.3 (page 34)

Comment by Prof. Dr. Gelbke

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

With regard to the available data, a set of recent in vivo genotoxicity studies of cytogenetic effects of TM clearly showed that TM is neither clastogenic nor aneugenic in somatic cells and also in germ cells in vivo.

Therefore, cat 1B is not justified and even cat 2 could be discussed controversially. Nevertheless, cat 2 is proposed as conservative classification. Additional relevant publications are provided herewith. For details please refer to Gelbke (2018b).

The following attachments are provided with this comment:

1) R. Barale, C. Scapoli, C. Meli, D. Casini, M. Minunni, A. Marrazzini, N. Loprieno, I. Barrai (1993). Cytogenetic effects of benzimidazoles in mouse bone marrow. Mut. Res. 300: 15-28 ("Barale _1993.pdf")

2) Capriglione, T, De lorio, S, Gay,F, Capaldo,A, Vaccaro, MC, Morescalchi, MA, Laforgia.V (2011). Genotoxic effects of the fungicide thiophanate methyl on Podarcis sicula assessed by micronucleus test, comet assay and chromosome analysis. Ecotoxicology 20: 885-891. ("Capriglione _2011.pdf")

3) CSGMT (1988). Strain differences in the micronucleus test. Mut. Res. 204: 307-316

("CSGMT _1988.pdf

4) Makita, T, Hashimoto, Y, Noguchi, T (1973). Mutagenic, Cytogenetic and Teratogenic Studies on Thiophanate-methyl. Toxicol. Appl. Harmacol. 24: 206-215 ("Makita _1973.pdf")

5) Salamone, MF, Mavournin, KH (1994). Bone marrow micronucleus assay: a review of the mouse stocks used and their published mean spontaneous micronucleus frequencies. Environ. Molecular Mutagen. 23: 239-273 ("Salamone+Mavournin _1994.pdf")

6) Gelbke, HP (2018b): Thiophanate methyl (TM): classification for genotoxicity (Gelbke 2018b mutagenicity of thiophanate methyl.pdf")

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mutagnicity comments Gelbke.zip

Dossier Submitter's Response
Please refer to comment 17.
RAC's response
Please refer to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	17

Comment received

Reference: CLH Report section 10.8.3 (page 34)

Applicant's comment (2 of 2):

With regard to the conclusion of the DS (KEMI) on the genotoxicity of Thiophanate-methyl (TM), several key aspects, including new data, should be considered by the RAC members. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018b).

Considering the complete available data package and the WoE, TM should arguably not be subject to classification for genotoxicity. The current classification with Muta 2 is still highly conservative but acceptable. The available data provide only a weak evidence for aneugenicity confined to effects seen in somatic cells, while new and reliable in vivo genotoxicity studies on germ cells demonstrated a lack of genotoxicity. A classification with Muta 1B is thus not acceptable, and the classification should take into account all reliable data available.

The following attachments are provided with this comment (submitted in two batches, batch 2 of 2):

1) New studies demonstrating adequate exposure for the ICR and the B6D2F1 mouse strains:
- TK study in ICR mice („Kuroiwa_2017_NIP_513-001_RD-10374.pdf")
- TK study in B6D2F1 mice („Kuroiwa_2018 _NIP_513-002_RD-10431.pdf")

- Analytical reports for the TK studies („Akai_2017_NIP_433-022_RD-10209.pdf; "Akai_2018_NIP_433-023_RD-10408.pdf)

2) New studies demonstrating a lack of clastogenic activity for the B6D2F1 mouse strain:
- Chromosome aberration assay in somatic cells in B6D2F1 mice: pretest („Aoto_2018_NIP_557-020_RD-10409.pdf")
- Chromosome aberration assay in somatic cells in B6D2F1: main test („Kuboki_2018_NIP_557-019_RD-10440.pdf")

3) OECD Tier 2 summaries for all new studies submitted with this comment:
- OECD tier 2 summaries
(„New studies Thiophanate-methyl MUTA, TK - tier 2 summaries.pdf")

4) The Collaborative Study Group for the Micronucleus Test (CSGMT), Strain difference in the micronucleus test, Mutat. Res., 204 (1988) 307-316 ("CSGMT, 1988.pdf")

5) Briese, B.H., Harder, V., Heidemann, A. (2018b): Mutagenicity of Thiophanate-methyl ("Briese et al_2018b.pdf")

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Mutagenicity comments SCC_2.zip

Dossier Submitter's Response

Nippon Soda Co. Ltd. has submitted two new toxicokinetic studies in which thiophanate-methyl was administered orally to mice by single administration. One of the studies was performed in B6D2F1/Crl mice and the other was performed in Crl:CD-1 (ICR) mice. In both studies similar results were obtained demonstrating exposure of plasma and testes to thiophanate-methyl and its metabolites. The concentrations of thiophanate-methyl were comparable in plasma and testes, while the concentrations of metabolites, especially carbendazim, in testes where lower than those in plasma. Based on these results the DS concludes that the systemic exposure is similar in B6D2F1/Crl mice and Crl:CD-1 (ICR) mice. Consequently, these results show that toxicokinetic differences could not explain a possible difference in sensitivity between B6D2F1/Crl mice and Crl:CD-1 (ICR) mice when used for detecting genotoxic effects of thiophanate-methyl.

However, it is generally considered that differences in toxic effects between strains of a species are only partly explained by toxicokinetic factors. The influence of toxicodynamic factors is commonly believed to be of equal significance. Due to varying test conditions (explained below) the available studies do not allow to make a consistent analysis of a possible difference in sensitivity between B6D2F1/Crl mice and Crl:CD-1 (ICR) mice regarding genotoxic effects and, accordingly, no assumptions about differences in the influence of toxicodynamic factors can be made.

Nippon Soda Co. Ltd. has also submitted a new oral bone marrow chromosome aberration study in B6D2F1/Crl mice to investigate the clastogenic potential of thiophanate-methyl performed according to OECD TG 475 and a new preliminary oral bone marrow chromosome aberration study in B6D2F1/Crl preceding the main study above. The preliminary study did not comply with the requirements of OECD TG 475. In the main study no increase in metaphases with structural chromosome aberrations were observed in mice treated with thiophanate-methyl. The DS therefore concludes that the result of the study does not support that thiophanate-methyl has clastogenic properties. The

observations in the preliminary study do not contradict the result of the main study. In the reports of the two studies it is also noted that no increase in polyploid cells was observed. Regarding this the DS would like to stress that the mammalian bone marrow chromosome aberration test (OECD TG 475) is not designed to measure aneuploidy and therefore it is not appropriate to conclude on this type of effect from results obtained with this test.

Assuming that B6D2F1/Crl mice and Crl:CD-1 (ICR) mice are equally sensitive to thiophanate-methyl, the earlier studies performed in Crl:CD-1 (ICR) mice that in the CLH report was considered to be of low reliability could now be included in the evaluation of the genotoxic potential of thiophanate-methyl. Furthermore, the view of the DS is that, despite the similar proportions of centromere-negative micronuclei obtained for thiophanate-methyl and the known clastogenic substance mitomycin C in B6D2F1 mice (RAR Vol 3 B.6.4.2.1 Doc. No. 557-007), the new negative bone marrow chromosome aberration study in B6D2F1/Crl mice should be ascribed higher weight in the analysis of clastogenicity, since this test is specifically designed to detect structural chromosome aberrations. Taking into consideration the results of the new studies, there is no longer support for a clastogenic effect of thiophanate-methyl. In addition, due to the negative results in the spermatogonial chromosomal aberration study in Crl:CD-1 (ICR) mice (RAR Vol 3 B.6.4.3.2 Doc. No. RD-03956 (557-012)) and the micronucleus test in germ cells in Crl:CD-1 (ICR) mice (RAR Vol 3 B.6.4.3.3 No. RD-10093 (557-017)), the DS considers that classification in Muta 1B would no longer be warranted.

The DS would like to highlight that uncertainties regarding a possible difference in sensitivity between B6D2F1/Crl mice and Crl:CD-1 (ICR) mice regarding genotoxic effects have to be considered in the evaluation. The reason why a consistent analysis of a possible difference in sensitivity between the strains cannot be made is because the testing conditions varied between studies. In the positive bone marrow micronucleus study in B6D2F1/Crl mice one treatment was used, while two treatments were used in the negative micronucleus study in Crl:CD-1 (ICR) mice (this is the only study in which two treatments were used). Hence, the treatment schedules used are not comparable. It is possible that repeated treatments result in a toxicokinetic behaviour that differs from that following single dosing. The available data does not allow for any firm conclusions on the behavior of thiophanate-methyl in mice during high repeated dosing.

Induction of structural chromosome aberrations was investigated in the negative bone marrow chromosome aberration study in B6D2F1/Crl mice and in the positive bone marrow micronucleus study in B6D2F1/Crl mice (the test method can discriminate between clastogenicity and aneugenicity). Hence, different test methods were used to measure clastogenicity. To convincingly demonstrate whether there is a difference in sensitivity or not between the two mouse strains regarding induction of structural and numerical chromosome aberrations, new studies would be required. The first study would be a bone marrow micronucleus study in Crl:CD-1 (ICR) mice using single treatment and, in case this study would be negative, an identically performed bone marrow micronucleus study in B6D2F1/Crl mice using single treatment would be required. However, it has to be considered if it would be ethically justified to perform these studies, since results from several animal studies are already available. In this respect, a crucial point is if it would be possible to make a reliable conclusion based on the available data, which takes the uncertainties described above into consideration.

The DS believes that there is sufficient information available to make a reliable conclusion about the genotoxicity of thiophanate-methyl. Overall, the DS concludes that the

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

RAC's response

The reason of the conflicting results reported in the two mouse strains used for MN assays has not yet been clarified. The similar distribution of thiophanate-methyl reported in the two strain indicates that there is no clear toxicokinetic explanation and that the reason of the possible different sensitivity could be related to other reasons (e.g. toxicodynamic factors, as suggested by the DS). Anyway, there is no reason to question the reliability of the positive result reported in B6D2F1/Crl mice as the evident and dose related increase in micronuclei observed at both sampling times provide a clear evidence of an *in vivo* mutagenic effect that cannot be disregarded.

While the analysis of the structure-activity relationship would indicate a possible DNA-reactivity of thiophanate-methyl , the available experimental results suggest that the MN induction reported both *in vitro* and *in vivo* is due to aneugenic activity. The only indication of clastogenic activity would derive from the results of centromeric staining conducted with the in vivo MN study in B6D2F1/Crl mice, but this indication is considered weak also by the authors of the study, whom in fact considered inconclusive the qualitative analysis of the molecular mechanism of MN induction.

Overall, the available data indicate that thiophanate-methyl is able to induce aneugenicity *in vitro* and *in vivo*. thiophanate-methyl is systemically available and was detected in gonads of mice at concentrations close to those found to be effective *in vitro*. In conclusion, the data are considered to indicate "the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells". It should also be considered that the major metabolite carbendazim, a recognized aneugenic substance, is already classified as Muta. 1B; H340. However, RAC concludes that classification of thiophanate-methyl as muta 1B is not warranted, as discussed in the opinion, and that category 2 is more suitable.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	18

Comment received

Reference: CLH Report section 10.8.3 (page 34)

Applicant's comment (1 of 2):

With regard to the conclusion of the DS (KEMI) on the genotoxicity of Thiophanate-methyl (TM), several key aspects, including new data, should be considered by the RAC members. Additional new studies and relevant publications are provided herewith. For details please refer to Briese et al. (2018b).

Considering the complete available data package and the WoE, TM should arguably not be subject to classification for genotoxicity. The current classification with Muta 2 is still highly conservative but acceptable. The available data provide only a weak evidence for aneugenicity confined to effects seen in somatic cells, while new and reliable in vivo genotoxicity studies on germ cells demonstrated a lack of genotoxicity. A classification with Muta 1B is thus not acceptable, and the classification should take into account all

reliable data available.

The following attachments are provided with this comment (submitted in two batches, batch 1 of 2):

1) New studies demonstrating adequate exposure for the ICR and the B6D2F1 mouse strains:
- TK study in ICR mice („Kuroiwa_2017_NIP_513-001_RD-10374.pdf")
- TK study in B6D2F1 mice („Kuroiwa_2018 _NIP_513-002_RD-10431.pdf")
- Analytical reports for the TK studies („Akai_2017_NIP_433-022_RD-10209.pdf; "Akai_2018_NIP_433-023_RD-10408.pdf)

2) New studies demonstrating a lack of clastogenic activity for the B6D2F1 mouse strain:
- Chromosome aberration assay in somatic cells in B6D2F1 mice: pretest („Aoto_2018_NIP_557-020_RD-10409.pdf")
- Chromosome aberration assay in somatic cells in B6D2F1: main test („Kuboki_2018_NIP_557-019_RD-10440.pdf")

3) OECD Tier 2 summaries for all new studies submitted with this comment:
- OECD tier 2 summaries
(„New studies Thiophanate-methyl MUTA, TK - tier 2 summaries.pdf")

4) The Collaborative Study Group for the Micronucleus Test (CSGMT), Strain difference in the micronucleus test, Mutat. Res., 204 (1988) 307-316 ("CSGMT, 1988.pdf")

5) Briese, B.H., Harder, V., Heidemann, A. (2018b): Mutagenicity of Thiophanate-methyl ("Briese et al_2018b.pdf")

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Mutagenicity comments SCC_1.zip

Dossier Submitter's Response
Please refer to comment 17.
RAC's response
Please refer to comment 17.

Date	Country	Organisation	Type of Organisation	Comment number
12.06.2018	Germany	BASF SE	Company-Downstream user	19

Comment received

We do not support the DS's conclusion and the classification proposal as Muta. 1B.
The presentation and evaluation of the available genotoxicity studies in the CLH-report appears imbalanced and is not using a weight of the evidence approach as recommended in Regulation (EC) 1272/2008. Studies not fitting to the DS's surprising hypothesis that Thiophanate-methyl (TPM) is clastogenic are either not mentioned in the discussion or discounted because of formalistic rather than scientific arguments.
The DS considers TPM as clastogenic because of 2 studies: the in vivo micronucleus test

of Proudlock (1999) in bone marrow of B6D2F1 mice and a literature study in lizards measuring genotoxic activity in a non-validated test system (Capriglione et al 2011). All guideline compliant GLP studies submitted by the notifier for Annex I renewal were called unreliable because "no data on target-cell exposure were presented" and "there is reason to be concerned about the sensitivity of this strain" (CRL:CD-1 (ICR)).
We cannot follow this line of argumentation because of the following aspects:
A) Discounting of mutagenicity studies in CD-1 mice because of lack of exposure
Negative micronucleus tests in bone marrow of CD-1 mice are discounted because of missing "evidence of bone marrow exposure". Plasma exposure to TPM was however demonstrated in the study in B6D2F1 mice of Proudlock (1999; RAR B.6.4.2.1). Thus, it was shown that TPM reaches the systemic circulation after oral exposure in mice under exposure conditions representative for the mouse bone marrow micronucleus test. Moreover, in a study on the biotransformation of radiolabeled TPM in CD-1 mice (RD-73073 and RD-73075; cf. section B.6.1.1.2 of the RAR presented in Appendix 1 to the CLH report), exposure of both plasma and testes to radioactivity was demonstrated. Thus, there is conclusive evidence available from the already submitted data for the test species mouse and the use of a different strain is not expected to influence the bioavailability. Moreover, the notifier has performed an additional studies comparing exposure of plasma and testes to TPM and MBC after oral exposure (Kuroiwa 2017 and Kuroiwa 2018) that will be made available to the RAC members in the course of commenting to the CLH report.
B) Sensitivity of the CD-1 mouse
The doubt of the DS regarding the sensitivity of strain CRL:CD-1 (ICR) is lacking a scientific rationale. The only argument provided is the apparent induction of micronuclei in the study of Proudlock in B6D2F1. Sufficient information is available from public literature confirming that the CD-1 mouse, which is the most widely used strain for bone marrow micronucleus test, is sufficiently sensitive to detect possible aneugenic and clastogenic effects of TPM (The Collaborative Study Group for the Micronucleus Test (CSGMT), Strain difference in the micronucleus test, Mutat. Res., 204 (1988) 307-316; Seiler J.P., The mutagenicity of benzimidazole and benzimidazole derivatives, Mutat. Res., 40 (1973) 339-348). Further evidence will be provided by the notifier during the commenting that both mouse strains have comparable target tissue exposure to TPM (Kuroiwa 2017 and Kuroiwa 2018). In addition, a chromosomal aberration study in the B6D2F1 mouse was clearly negative either.
C) Available evidence for a clastogenic effect
In a literature study of Capriglione TPM was tested in lizards at an undefined level. The test system used was not validated, the test animals captured in the wild, and neither the test substance used were specified nor the exposure levels. There is no experience with this type of test system and thus the results of the study can only be considered supportive information that would be clearly overruled by OECD Guideline studies on the same endpoints (clastogenicity and aneugenicity).
In the in vivo micronucleus study of Proudlock (1999; RAR B.6.4.2.1) in B6D2F1 mice additional centromere staining was performed to elucidate whether the slight increase in micronucleus frequency is caused by a clastogenic or aneugenic mechanism. In the high dose tested an overall incidence of only 6.3 micronuclei per 2000 immature erythrocytes per test animal was observed while for the positive control MDC the incidence was 24.4. Considering the low number of micronuclei for the TPM dose groups, a statistically meaningful analysis of centromere distribution is not possible. This is a common limitation of centromere staining when only weak increases of micronuclei are seen. The study of Proudlock is therefore not useful to draw any conclusion regarding the underlying mechanism of action. There is, however, convincing evidence from other GLP-compliant guideline studies that TPM is not clastogenic:

• No clastogenic potential was detected in an in vitro chromosomal aberration study (OECD 473) in CHO cells (Murli 1988, RAR B.6.4.1.3). No deficiencies were seen in this study according to the DS as stated in Appendix 1. This test system is considered highly sensitive for clastogenic effects. • Induction of micronuclei in vitro in human lymphocytes with positive centromere staining (Marshall 1996; RAR B.6.4.1.5). In this study a dose dependent increase of micronuclei was observed and accompanied by a dose-dependent increase in centromere positive micronuclei. This is also stated by the DS in the Appendix 1 but not mentioned in the discussion. This study should also be taken into account for the overall weight of evidence. • Negative in vivo chromosomal aberration data in the B6D2F1 mouse (Kuboki 2018) not yet included in the CLH report but to be submitted by the notifier as part of the commenting. No increase in structural chromosomal aberrations in mouse bone marrow is seen in this guideline and GLP-compliant study. This study confirms the result already seen in vitro and is the ultimate proof that TPM does not have any clastogenic potential in a test system considered highly sensitive. The DS proposes a classification as Mut 1B for TPM. We cannot follow this proposal because of the following aspects: ☐ TPM was negative in vitro for gene mutations in bacterial and mammalian systems. It did not induce chromosomal aberrations in CHO cells but showed aneugenic effects in human lymphocytes without metabolic activation. ☐ In vivo, it was confirmed in mouse bone marrow in B6D2F1 mice that TPM does not have a clastogenic effect. A weak increase in micronuclei in a study in B6D2F1 mice, even though statistically significant, was within the biological variation range of the mouse strain used and could not be confirmed at similar dose levels in CD-1 mice. ☐ With regard to germ cell mutagenicity TPM did not show any effect in a range of germ cell studies: no indication of an effect was seen in a dominant lethal test with deficiencies. No clastogenic potential was seen in a spermatogonial chromosomal aberration study in CD-1 mice (both in pre-test and main study) and no clastogenic or aneugenic potential was seen in a spermatid micronucleus test (both pre-test and main study). Exposure of the target tissue was confirmed in the above mentioned toxicokinetic experiments. Overall, the existing classification as Muta Cat 2 is considered highly precautionary based on the weak response seen in the micronucleus test of Proudlock at high concentrations. However, taking into account that an effect in germ cells could be clearly ruled out, it was sufficiently demonstrated that TPM does not share the properties of its metabolite MBC. Therefore, a classification as Muta. 1B is not warranted.
Dossier Submitter's Response
Please refer to comment 17.
RAC's response
Please refer to comment 17.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	20
Comment received				
While carbendazim, classified Repr. 1B H360FD, is a major metabolite of thiophanate-methyl in mammals. It is agreed that no classification is triggered based on studies performed with thiophanate-methyl.				

Dossier Submitter's Response
Noted, thank you.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	21

Comment received

As carbendazim is a major metabolite of Thiophanate-methyl and this substance is classified as Repr. 1B, information available for carbendazim should be considered as relevant and discussed here.

Dossier Submitter's Response

As there are negative studies on reproductive toxicity with thiophanate-methyl in rats and rabbits available, the DS considers that 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, see tables below. The studies on thiophanate-methyl itself are therefore be considered to be the most relevant for classification of thiophanate-methyl.

Table B.6.1.4-2: Proportions of radioactive substance (% of peak area) in test substance samples (human microsomes)

Substance	Incubation time (h)			
	0	**0.5**	**1**	**2**
TM	93.81	91.90	89.71	85.09
4-OH-TM	nd	0.73	0.75	0.89
MBC	1.01	3.12	3.96	5.05
5-OH-MBC	nd	0.43	1.17	4.55
IM-1*1	1.60	1.60	1.53	1.53
IM-2*1	1.88	1.39	1.94	1.17
IM-3*1	1.70	0.41	nd	nd
UM*2	nd	0.42	0.94	1.71
Total	100.0	100.0	100.0	100.0

*1 Impurities in stock solution of the tested substance
*2 Unknown metabolite
nd: Not detected

Table B.6.1.4-3: Proportions of radioactive substance (% of peak area) in test substance samples (rat microsomes)

Substance	Incubation time (h)			
	0	**0.5**	**1**	**2**
TM	93.54	84.66	80.60	72.73
4-OH-TM	nd	1.82	2.49	3.81
MBC	0.90	6.63	6.43	4.53
5-OH-MBC	nd	2.93	7.35	15.57
IM-1*1	1.78	1.77	1.31	1.63
IM-2*1	1.75	1.09	1.08	0.79
IM-3*1	2.04	0.40	nd	nd
UM*2	nd	0.71	0.75	0.94
Total	100.0	100.0	100.0	100.0

*1 Impurities in stock solution of the tested substance
*2 Unknown metabolite
nd: Not detected

The following was concluded at the EFSA Expert meeting for TM:

"*Although it is acknowledged that thiophanate-methyl produces the metabolite carbendazim that is classified as Repro cat 1B, and therefore a concern cannot be completely excluded, the majority of the experts agreed that the data base is sufficient for excluding classification of the substance regarding toxicity for reproduction*."
RAC's response
The rapporteur agrees with the DS conclusion.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	22
Comment received				
As ATE value for the classification as Acute Tox. 4, H332 the LC50 value of 1.7 mg/l/4h should be added in the column "specific Conc, Limits, M-factors, ATE" in Table 6.				
Dossier Submitter's Response				
Agreed. See also comment 2.				
RAC's response				
Agreed.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	23
Comment received				
STOT RE2 is supported based on the data presented. For the hazard statement of classification the respective organ should be specified: H373: May cause damage to the thyroid through prolonged or repeated exposure.				
Dossier Submitter's Response				
Agreed.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	Netherlands		MemberState	24
Comment received				
Specific Target Organ Toxicity after Repeated Exposure (STOT RE) Page no. 57 - 66 The thyroid size is increased in a clearly dose dependent manner at or above the guideline criteria for STOT RE 2, while some limited size increases are seen in a few studies also at dose levels within the criteria for STOT RE 2. A mere increase in organ size (hypertrophy/hyperplasia) is not a sufficiently adverse effect warranting classification for STOT RE. The criteria allow classification when a relevant related mechanism is demonstrated or relevant secondary effects/organ dysfunction is demonstrated. In this case, thiophanate-methyl inhibits TPO resulting in reduced thyroid hormone levels that has an effect on thyroid size. It is thus considered that the thyroid size is a result from thyroid hormone inhibition. However, mere increased thyroid size can be seen as a successful adaptive mechanism without further toxicological relevant consequence. Only when the adaption falls short and subsequent more severe effects arise this would				

warrant classification for STOR RE according to the CLP criteria. Therefore we do not support the proposed classification of thiophanate-methyl as STOT-RE 2 (H373). Notably, the mortality observed starting at 150 mg/kg bw/day in the rabbit developmental toxicity range finding study (dosed for 14 days) does not seem as relevant for classification for STOT RE because of the nature of the study (pregnant dams), low mortality incidence without a clear dose/time-dependent relationship. Also due to the short nature of developmental tests, it is unclear whether this should be considered acute toxicity or repeated dose toxicity. Is there information on which day the rabbits died?
Dossier Submitter's Response
In addition to hypertrophy and increased weight, hyperplasia was seen in two rat studies below the Gudiance value. At higher doses this progressed to tumours and effects were seen on thyroid hormones in several studies. These effects are considered severe and to justify classification in STOT RE 2 (H373). As stated in the CLH dossier: "In rabbits, mortality was observed at 150 (1 animal), 300 (1 animal) and 600 (2 animals) mg/kg bw/day in a developmental toxicity study (RAR Vol 3 B.6.2.2.2). Exposure in this study was for 14 days (GDs 6-19) and mortality occurred on days 19 (150 mg/kg bw/day), 24 (300 mg/kg bw/day), 20 and 23 (600 mg/kg bw/day). It is therefore considered an effect of repeated administration of the test substance rather than an acute effect.".
RAC's response
The rapporteur agrees with the DS conclusion.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	25
Comment received				
Point 10.12.3 STOT RE page 62: The proposal for classification STOR RE 2 H373 (mortalities in pregnant rabbit and thyroid effects) is agreed upon.				
Dossier Submitter's Response				
Noted, thank you.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
26.06.2018	United Kingdom		MemberState	26
Comment received				
Thiophanate-methyl (ISO) (EC: 245-740-7; CAS 23564-05-8) We agree that the substance is not rapidly degradable and the aquatic degradant carbendazim is relevant for hazard classification. Aquatic ecotoxicity data for carbendazim is presented in the CLH which indicates the degradant may be more ecotoxic than the parent. However, important data is not available to confirm the validity of these studies. Carbendazim, acute toxicity to Ictalurus punctatus [Report A30119, xxxx, 1984]:				

The static test used Ictalurus punctatus (Channel catfish) and there are no details for the exposure concentration range, test system media, treatment preparation or test item stability over study period. Also, the DS notes that control raw data are missing and study was run prior to GLP. We consider further information is required to consider these issues and the validity of the study. This is important as the endpoint is presented as the most sensitive acute endpoint for classification. Please can you provide further details to support the reliability of the study endpoint and if available test guideline validity criteria.

In addition, it is unclear if the test species is relevant as it is not a recognised fish species for regulatory ecotoxicity testing purposes (i.e. it is not listed in standard OECD fish test guidelines) and its relative sensitivity to other standard test fish species is unknown. Also the reproducibility of this non-standard test is unknown. Please can you provide further information to support the use of this test species considering these points.

Carbendazim, acute toxicity to Daphnia magna [Fischer, 1988]:
The CLH report does not include an assessment of relevant test guideline validity criteria and if these were met as has been undertaken for the study with the parent substance thiophanate-methyl. Please can you provide this information to support the validity of the study.

It appears that the results of 3 out the 4 test systems were disregarded due to analytical contamination and 'varying results'. It is unclear if the remaining test system C has analytical support and if so, what the results were. Please can you provide analytical support to justify the use of the nominal endpoint.

Carbendazim, acute toxicity to Oncorhynchus mykiss [Report A52914, xxxx 1976]:
The study was not conducted to GLP or a recognised validated test guideline. It is unclear if analytical verification is available to support the use of the nominal endpoint. Please can you provide further information to support the reliability of the test such as details of comparison with current test guideline validation criteria and analytical verification if available.

Please can you explain why the endpoint was recalculated instead of using the study value.

Carbendazim, toxicity to Oncorhynchus mykiss [Report A40788, xxxx 1976]:
The study followed test guideline OECD 204. We note that the OECD 204 test guideline was withdrawn by the OECD in April 2014 and it is not suitable to determine long term endpoints. On this basis, a chronic toxicity to fish endpoint for carbendazim is not available and it would be useful to consider the surrogate approach depending on the validity of acute toxicity endpoints.

Carbendazim, toxicity to Daphnia magna [Kelly et al, 1997]:
Table 160 presents the 21-d NOEC based on nominal concentrations. The text in the study description indicate the endpoint is based on measured concentrations. Please can you confirm the basis as either nominal or measured.

The study followed OECD 202 (1984). It would be useful if the DS provided an assessment of the study against current test guideline validity criteria as this has been undertaken for the chronic Daphnia study with the parent substance thiophanate-methyl.

We note that the study followed OECD 202. It would be useful to consider if an acute

toxicity to Daphnia endpoint and analytical data are suitable to support the Fischer, 1988 endpoint. Therefore, please can you provide an acute 48-h EC50 if available.
Dossier Submitter's Response
Carbendazim is a metabolite of Thiophanate-methyl but also an active ingredient by itself, and the studies on this substance were not re-evaluated by the DS but were referred to the RAR and EFSA conclusion for Carbendazim (published in 2010, Germany was the designated RMS under 1107/2009). There is also a recent CLH dossier available for this compound.
RAC's response
RAC agrees that the substance is not rapidly degradable and that carbendazim information is relevant for hazard classification of Thiophanate-methyl. RAC agrees that for the *I. punctatus* the summary provided lacks of relevant data. However, the DS gave a reliability of 2 to the test in the carbendazim dossier. The study was performed according to ASTM Guideline. RAC also considers that the species and size used is relevant for acute toxicity. This species is used in USEPA Guidance 850.1075 Fish Acute Toxicity Test, Freshwater and Marine. The study is therefore relevant for classification. RAC agrees that the information provided for the Fischer study (1988) does not allow to proof validity criteria. However, this study is also included in the carbendazim dossier where the test was given reliability 2 and it can be seen that validity criteria are met: 1. in the control, not more than 10 % of the daphnia should have been immobilised or trapped at the surface of the water. 2. The dissolved oxygen concentration at the end of the test should be 60 % 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. RAC agrees that information is missing to independently proof the reliability, reasons for recalculation and validity of the test done with *O. mykiss* (Report A52914, xxxx 1976). The test does not follow any guideline and is non GLP. However, the DS considered it valid. The study will be used as supporting information. RAC agrees, OECD TG 204 cannot be considered a suitable long-term test as indicated in Guidance on Information Requirements and Chemical Safety Assessment. In relation to the chronic daphnia study, Kelly *et al*. (1997), RAC understands that the endpoint is based on measured concentration. In the carbendazim dossier this study is also included. There, it can be seen that the mortality of the parental generation in the control was 10 % at the end of the test and meeting 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 48h EC_{50} for this test is not provided, however RAC considers the acute for daphnia valid. In addition, in this test daphnias 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.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON THIOPHANATE-METHYL (ISO); DIMETHYL (1,2-PHENYLENEDICARBAMOTHIOYL)BISCARBAMATE; DIMETHYL 4,4'-(O-PHENYLENE)BIS(3-THIOALLOPHANATE)

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Germany	Nippon Soda Co. Ltd.	Company-Manufacturer	27

Comment received

CLH Report, chapter 11.7.1 Acute aquatic hazard

Page 200: The applicant does not agree to the proposed classification as Aquatic Acute 1 (H 400) with M-factor = 10 based on the lowest endpoint for Carbendazim (0.019 mg/L for fish).

Thiophanate-methyl is not readily biodegradable and degradation is mainly caused by hydrolysis. Thiophanate-methyl is stable to hydrolysis at ambient temperature (22 °C) and pH of 5 – 7; hydrolytically degraded at higher pH. The DT50 for Thiophanate-methyl: 46.8 days (pH 7, 22 °C) and 1.0 days (pH 9, 22 °C) [based on hydrolysis study by Soeda & Nomura, 1986].

The lowest endpoint for Thiophanate-methyl is 4.4 mg/L for daphnids obtained in an acute toxicity study over 48 hours. Thus, based on the DT50 value for Thiophanate-methyl at environmentally relevant pH values, the toxicity of Carbendazim is not relevant for the acute classification of Thiophanate-methyl. As all toxicity endpoints of Thiophanate-methyl are above 1 mg/L, no acute classification is considered necessary.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Carcinogenicity comments SCC_1.zip

Dossier Submitter's Response

Agree that thiophanate-methyl is not readily biodegradable and that degradation is mainly driven by hydrolysis. However biotic degradation may be expected to contribute to the degradation, since rapid degradation of the parent molecule (DT50 < 1 day, 20ºC) was observed also in test systems with pH value below 7 (study on soil; Voelkl, 2002).

Due to the rapid conversion of Thiophanate-methyl to Carbendazim (up to ca 80% in one week in aquatic test systems), the DS proposed that the toxicity of the metabolite is relevant for the classification. Hence, the M-factor was based on the acute toxicity of Carbendazim.

RAC's response

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.

Date	Country	Organisation	Type of Organisation	Comment number
20.06.2018	Germany		MemberState	28

Comment received

Page 131 point 11.5 Acute aquatic hazard, table 119 and page 172 point 11.6 long-term aquatic hazard, table 160:
We would recommend to summarize only valid studies and data of the active substance and its relevant metabolites since studies with preparations of the active substance (e.g. BAS 32510F or Topsin 500 SC) are not relevant for classification and labelling purposes of Thiophanate-methyl.

Dossier Submitter's Response
Only studies considered as valid are included in Table 119 and 160. Studies on preparations were included were considered by the DS as relevant for the assessment of the active ingredient.
RAC's response
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 TopsinM 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 endpoints obtained not relevant for classification. In the case of TopsinM WDG purity of the a.s. is > 70 %. However, in the test remains unclear the real effect of the active substance since it transforms to carbendazim. RAC also considers the endpoints obtained in this test not relevant for classification of the substance.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	29
Comment received				
FR would agree with the classification and the M-factor values proposed for Environmental hazards if the proposals of classification and M-factor could be based on data from a metabolite and not only on data of the substance. If the classification proposal has to be based only on toxicity data of the substance, the current classification proposed in the CLH report would have to be updated.				
Dossier Submitter's Response				
Noted. See also DS response to comment number 27.				
RAC's response				
Noted. RAC agrees with the DS in using carbendazim ecotoxicological data to classify the substance.				

PUBLIC ATTACHMENTS
1. Mutagnicity comments Gelbke.zip [Please refer to comment No. 16]
2. Carcinogenicity comments Gelbke.zip [Please refer to comment No. 8]

CONFIDENTIAL ATTACHMENTS
1. Mutagenicity comments SCC_2.zip [Please refer to comment No. 17]
2. Mutagenicity comments SCC_1.zip [Please refer to comment No. 18]
3. Haines_2018_Rat_TPO _NIP_593-004_RD-10591.pdf [Please refer to comment No. 9]
4. Haines_2018_Pig_TPO _NIP_593-003_RD-10590.pdf [Please refer to comment No. 10]
5. Haines_2018_Human_TPO _NIP_593-001_RD-10588.pdf [Please refer to comment No. 11]
6. Carcinogenicity comment SCC_2.zip [Please refer to comment No. 12]
7. Carcinogenicity comments SCC_1.zip [Please refer to comment No. 3, 13, 27]