Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-methyl-1,2-benzothiazol-3(2*H*)-one; [MBIT]

EC Number: -
CAS Number: 2527-66-4

CLH-O-0000001412-86-209/F

Adopted

8 June 2018

8 June 2018

CLH-O-0000001412-86-209/F

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

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

Chemical name: **2-methyl-1,2-benzothiazol-3(2*H*)-one; [MBIT]**

EC Number: **-**

CAS Number: **2527-66-4**

The proposal was submitted by **Poland** and received by RAC on **8 May 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Poland has submitted a Background Document (Annex I) containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **19 July 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 September 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tiina Santonen**

Co-Rapporteur, appointed by RAC: **Stephen Dungey**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-methyl-1,2-benzothiazol-3(2*H*)-one; [MBIT]	-	2527-66-4	Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 2	H331 H311 H301 H314 H318 H317 H400 H411	GHS05 GHS06 GHS09 Dgr	H331 H311 H301 H318 H317 H410		**inhalation**: ATE = 0.5 mg/L (dusts or mists) **dermal**: ATE = 300 mg/kg bw **oral**: ATE = 175 mg/kg bw M=1	
RAC opinion	TBD	2-methyl-1,2-benzothiazol-3(2*H*)-one; [MBIT]	-	2527-66-4	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1C Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 2	H312 H301 H314 H318 H317 H400 H411	GHS05 GHS06 GHS09 Dgr	H312 H301 H314 H317 H410	EUH071	**dermal**: ATE = 1100 mg/kg bw **oral**: ATE = 175 mg/kg bw Skin Sens. 1A; H317: C ≥ 0.0015% M=1	
Resulting Annex VI entry if agreed by COM	TBD	2-methyl-1,2-benzothiazol-3(2*H*)-one; [MBIT]	-	2527-66-4	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1C Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 2	H312 H301 H314 H318 H317 H400 H411	GHS05 GHS06 GHS09 Dgr	H312 H301 H314 H317 H410	EUH071	**dermal**: ATE = 1100 mg/kg bw **oral**: ATE = 175 mg/kg bw Skin Sens. 1A; H317: C ≥ 0.0015% M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier submitter (DS) did not propose classification for physical hazards. All of the relevant physico-chemical hazards were assessed according to test methods described in Part I of the UN Recommendations on the Transport of Dangerous Goods – Model Regulations (UN RTDG). Based on these results, the DS concluded that MBIT is not flammable, explosive, or oxidising. It can be considered as thermally stable at room temperature. No flash point was determined as the substance is a solid and does not have a melting point below 40 °C.

Comments received during public consultation

No comments were received regarding physical hazards.

Assessment and comparison with the classification criteria

There were no indications of physical hazards based on the test results available. MBIT is not explosive, oxidizing, flammable. Therefore, RAC agrees with the DS that **classification is not required for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify MBIT as Acute Tox. 3 by all routes of exposure; oral, dermal and inhalation.

For **acute oral toxicity**, there is one study in the Background Document (Annex I) that was performed in the Wistar rat (females) according to the OECD Test Guideline (TG) 425. There were deviations to the OECD TG (low temperature: 14–26 °C; low humidity: 0–70 %, no average provided) that probably did not affect the results. Therefore, this study was considered by the DS to be reliable with restrictions. The doses tested were 55, 175, 550 and 2 000 mg/kg bw. Deaths occurred on day 1 in the three highest dose groups: 1/3 rats died at 175 mg/kg bw, 2/2 at 550 mg/kg bw and 1/1 at 2 000 mg/kg bw. Clinical signs included lethargy, piloerection, ataxia, prostration, flaccid muscle tone, negative righting reflex, few faeces, tremors, wetness of the nose/mouth area and laboured breathing. Necropsy revealed abnormalities of the thymus, kidneys, liver and gastrointestinal tract. The oral LD_{50} in the rat (female) was calculated as 175 mg/kg bw (95 % confidence interval 54-608 mg/kg bw) using the AOT425 statistical program provided by the US EPA. Therefore, the DS proposed classification as Acute Tox. 3; H301. Furthermore, the DS proposed an Acute toxicity estimate (ATE) value of 175 mg/kg bw.

For determination of **acute inhalation toxicity**, pure MBIT could not be tested due to unspecified physical chemical properties of the technical material. Therefore, testing was performed using a commercial product with a formulation of 24 % MBIT in 96 % propylene glycol 400. Acute inhalation toxicity was tested in Sprague-Dawley rats (5 males and 5 females)

according to the OECD TG 403 and US EPA 870.1300. The animals were exposed to the test substance in the form of aerosol during 4 h, nose only. The study was performed in accordance with GLP, and there were no deviations from the guideline. Only one dose level was tested, 2.22 mg/L of the product, thus 0.53 mg MBIT/L.

There was no mortality during the study. Clinical signs included activity decrease, piloerection and respiratory chirps, which were no longer evident by day 8. Four animals lost or failed to gain weight during the first week. There were no observable abnormalities in the gross necropsy. LC_{50} was therefore > 2.22 mg/L product and > 0.53 mg/L MBIT. As an exact LC_{50} value could not be determined, the DS proposed to derive an estimated value for the inhalation route from Table 3.1.2 of the CLP Regulation, and proposed to classify MBIT as Acute Tox. 3; H331 for inhalation. Based on the same Table, they also proposed a converted acute toxicity point estimate of 0.5 mg/L to be used in the calculation of the ATE for classification of mixtures (0.5 < Category 3 ≤ 1.0).

For **acute dermal toxicity**, two studies were included in the CLH report. Both were performed in the rat (Wistar and Fisher 344, both sexes) according to the OECD TG 402, and considered reliable by the DS. Purity of the test compound was ≥ 98.34 % in both studies. However, the results of these studies differed from each other. In the first study in Wistar rats, all ten rats died within one day of dosing at 5 000 mg/kg bw MBIT (dermal application). Also at 2 000 mg/kg bw, 3/5 females died by day 1. In the interest of conserving animals, particularly since the test material is a strong irritant, no further dosing was conducted in this study. At 5 000 mg/kg bw, lethargy, ataxia and tremors were noted in one rat. At necropsy, observations included abnormalities of treated skin, red areas on thymus, red areas on pancreas, yellow staining of the fatty tissue in the peritoneal cavity posterior to the kidney and intestinal abnormalities. At 2 000 mg/kg bw, the clinical observations included wetness of anogenital area and chromodacryorrhea (excretion of porphyrin). At necropsy, abnormalities included the treated skin (erythema, oedema, eschar and flaking), the pancreas, the thymus and the gastro-intestinal tract. In the surviving rats at 2 000 mg/kg bw, body weight gains were normal. The study director judged from these findings that the dermal LD_{50} would probably be slightly below 2 000 mg/kg bw, and that MBIT would be classified in category 2 for Acute Tox. (200 mg/kg bw < dermal LD_{50} ≤ 2 000 mg/kg bw).

In the second study in Fisher 344 rats, there were no deaths at either the 2 000 or 5 000 mg/kg bw dose levels. At 5 000 mg/kg bw, there was dermal irritation (erythema, oedema, eschar, desquamation, blanching and/or hyperkeratosis) in several animals of both sexes. No further signs of toxicity were observed in any of the animals. In both groups, all animals lost weight by day 1, but apart from one female at 2 000 mg/kg bw, and one female and one male at 5 000 mg/kg bw, all three of which returned to their initial body weights, all animals gained weight by the end of the 14-day observation periods. In this study, the LD_{50} for the dermal route was considered > 5 000 mg/kg bw.

As there is no exact LD_{50} value for the dermal route but it was judged to be above 200 and below 2 000 mg/kg bw, the DS proposed to derive an estimated value for the dermal route from Table 3.1.2 of the CLP regulation. The converted acute toxicity point estimate is 300 mg/kg bw for classification of mixtures (200 < Category 3 ≤ 1 000). The DS therefore proposed to classify MBIT as Acute Tox. 3 for the dermal route, and an ATE of 300 mg/kg bw.

Comments received during public consultation

One Member State Competent Authority (MSCA) commented that the LC_{50} of > 0.53 mg/L is very close to the concentration limit for Cat. 2 of acute inhalation toxicity, and that as the study was performed with a formulation instead of the active substance, there are some uncertainties,

which is why classification in Cat. 2 instead of Cat. 3 should be discussed. In addition, they recommended proposing an ATE value to facilitate uniform and reproducible classification of mixtures.

One MSCA supported classification as Acute Tox. 3 for the oral route. Regarding exposure via skin, they considered the estimated LD_{50} value to be closer to 2 000 mg/kg bw than below 1 000 mg/kg bw, because 3/5 animals died at the dose of 2 000 mg/kg bw, concluding that Acute Tox. Cat. 4 would be more appropriate than Cat. 3. In addition, they were of the opinion that classification for acute inhalation toxicity in Cat. 3 would not be justified, as there was no mortality or severe toxicity seen in the study.

There was one comment from the manufacturer. They were in agreement with the classification of MBIT as Acute Tox. 3 for the oral and dermal routes, but disagreed with the proposed classification of Acute Tox. 3 for the inhalation route. This was based on the physico-chemical properties of the technical material, which is reported as a crystalline yellow solid of very low vapour pressure, with a melting point above 50 °C. According to the comment, it is also due to these properties that acute inhalation testing was carried out on a formulation and not the active substance itself. They conclude that it would be impossible to create corresponding air concentrations under use conditions, and note that taking into account the mode of action, labelling as EUH071 might be more appropriate than classification as Acute Tox. 3; H331.

The DS replied to the comment from the manufacturer, pointing out that they have proposed to classify MBIT as Skin Corr. 1B, and that the inhalation study did not show corrosive effects on the respiratory tract. They therefore did not think that EUH071 would be necessary.

Assessment and comparison with the classification criteria

Oral route

There is only one study available in the dossier, performed according to the OECD TG 425 with deviations, and therefore evaluated by the DS to be reliable with restrictions. In this study, the LD_{50} value was calculated to be 175 mg/kg bw (95 % confidence interval 54-608 mg/kg bw). The criteria for Acute Tox. 3; H301 is $50 < LD_{50} \leq 300$ mg/kg bw. As 1/3 rats died at 175 mg/kg bw and 2/2 at 550 mg/kg bw, the deaths occurred already on day 1, and there were clear clinical signs as well as abnormalities in the necropsy. RAC agrees with the DS that classification as **Acute Tox. 3; H301** for the oral route is warranted for MBIT with an **ATE of 175 mg/kg bw** bw for the oral route for classification of mixtures.

Inhalation route

There was one study available for acute inhalation toxicity, where only one concentration of the product, not the active substance, was tested. However, the study was conducted according to the OECD TG 403 and US EPA TG 870.1300 under GLP; there were no deviations and it was therefore considered reliable without restrictions by the DS. The LC_{50} was found to be > 0.53 mg (MBIT)/L, the only tested concentration, which corresponds to the 24 % MBIT in the formulation. The criteria for Acute Tox. 3; H331 is $0.5 < LC_{50} \leq 1.0$ mg/L (mist). The dose level tested is at the low end of the criteria for Acute Tox. 3, but as there was no mortality or clear signs of toxicity seen at this dose level, RAC is of the opinion that there are no justifications for classification of MBIT as Cat. 3 for the inhalation route. Furthermore, as this was the only dose level tested, it is not possible to evaluate whether classification as Acute Tox. 4 would or would not be appropriate. Therefore, RAC proposes **no classification due to lack of data**.

However, RAC is of the opinion that MBIT should be labelled with EUH071 ("Corrosive to the respiratory tract"). In the criteria, it is stated that EUH071 applies "For substances and mixtures in addition to classification for inhalation toxicity, if data are available that indicate that the

mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I. For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled."

Based on the result of the skin corrosion/irritation test, it is evident that MBIT has corrosive properties and may therefore also be a respiratory tract corrosive/irritant.

While in the acute inhalation study there were no clear signs of toxicity or observable abnormalities in the gross necropsy related to respiratory corrosivity/irritation, the clinical signs, although reversible, included decreased activity, piloerection and respiratory chirps, the latter indicating a respiratory response to MBIT after an acute exposure. Importantly, MBIT was only tested at one concentration (0.53 mg MBIT/L), which did not allow to conclude on the acute inhalation toxicity of MBIT. Therefore, no acute inhalation toxicity classification was proposed due to lack of data, not because no toxicity was seen, and Note 1 (Table 3.1.3) is considered to apply: MBIT is skin corrosive, and no conclusive acute inhalation test data are available. Although MBIT is not volatile, it may be inhaled in aerosol form in exposure scenarios in which aerosols are formed.

In conclusion, RAC is of the opinion that labelling MBIT with **EUH071 ("Corrosive to the respiratory tract")** is warranted. This would also be consistent with the classification for other isothiazolinones.

Dermal route

There were two studies on acute toxicity via the dermal route in the dossier. Both were performed according to the OECD TG 402 and considered reliable without restrictions by the DS. Mortality was seen in the study: by day 1, 10/10 of the rats died at 5 000 mg/kg bw. Furthermore, at 2 000 mg/kg bw, 3/5 of the females died, also by day 1. In the interest of conserving animals, particularly since the test material is a strong irritant, no further dosing was conducted in this study. Therefore, the LD_{50} was considered to be slightly below 2 000 mg/kg bw based on the result in females. In the second study, no mortalities or signs of toxicity were seen either at 2 000 mg/kg bw or 5 000 mg/kg bw, apart from skin irritation at the higher dose. The LD_{50} was therefore determined to be > 5 000 mg/kg bw. There is not enough information in the dossier to explain the different outcomes of these two studies. Both were performed in the rat (first in Wistar, second in Fisher 344), and both sexes were included in both studies, except for the 2 000 mg/kg bw dose in Wistar, where only females were included (dosing was discontinued following mortality in females).

The criteria for Acute Tox. 3; H311 is 200 mg/kg bw < LD_{50} ≤ 1 000 mg/kg bw, and for Acute Tox. 4; H312 the criteria is 1 000 < LD_{50} ≤ 2 000 mg/kg bw. Based on the available information, there are no data indicating that the LD_{50} would be below 1 000 mg/kg bw, and therefore Cat. 3 cannot be justified. However, in one of the two studies, the LD_{50} was below 2 000 mg/kg bw in females. Therefore, RAC concludes that MBIT should be classified as **Acute Tox. 4; H312** via the dermal route with a converted **ATE of 1 100 mg/kg bw** for classification of mixtures (Table 3.1.2 of the CLP Regulation).

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

Summary of the Dossier Submitter's proposal

The DS did not propose classification, as there was no toxicity to a specific organ in the absence of lethality observed in the acute oral, inhalation and dermal toxicity studies, or there were no transient effects occurring after single exposure, especially for respiratory tract irritation and

narcotic effects (respectively). The DS noted that the guidance on CLP Criteria (Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017) states that it is reasonable to assume that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing respiratory tract corrosion. However, in the acute inhalation toxicity study in rats, there were no clinical signs indicating respiratory irritation. The gross necropsy did not reveal any observable abnormalities related to respiratory irritation either.

As additional information in relation to STOT SE 3 (respiratory tract irritation), the DS presented data from two respiratory depression (RD) studies conducted with two structurally related compounds: octyl isothiazolinone (OIT, CAS 26530-20-1) and methyl isothiazolinone (MIT, CAS 2682-20-4). The upper airway irritation RD_{50} values (exposure concentration producing a 50 % respiratory rate decrease) were calculated to be 19.9 µg/L for OIT and 157 µg/L for MIT. RD_{50} results are not available from MBIT. The DS did not propose to classify MBIT for STOT SE 3 noting that the upper airway irritation test is a measure of sensory irritation and whilst it can be used for setting up workplace exposure limits, it is not used for classification purposes.

Comments received during public consultation

No comments were received concerning specific target organ toxicity following single exposure.

Assessment and comparison with the classification criteria

According to the criteria, specific target organ toxicity (single exposure, STOT SE 1/2) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically addressed under other hazard categories are included. These adverse health effects produced by a single exposure include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism, and these changes are relevant for human health. Based on the data available, RAC concurs with the DS that a classification as STOT SE 1 or 2 is not warranted for MBIT.

Regarding STOT SE 3 (respiratory tract irritation), as already mentioned above, it is reasonable to assume that skin corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing respiratory tract corrosion. However, RAC is of the opinion that labelling MBIT with EUH071 ("Corrosive to the respiratory tract"), as proposed under acute toxicity, is more appropriate and adequately covers this hazard. Therefore, **classifying for STOT SE is not warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify MBIT as Skin Corr. 1B; H314. The dossier includes one study on skin corrosion/irritation, performed in the rabbit (New Zealand White, females) in compliance with OECD TG 404. There were no deviations from the guideline, and the study was considered reliable without restrictions. Initially, one rabbit was dosed 0.5 g of MBIT on the intact skin on the back at site 1, and MBIT was kept in contact with the skin for 3 minutes. Erythema and oedema were scored after one hour, and since the exposure did not indicate a corrosive effect, two additional rabbits were included. All three rabbits were dosed at site 2 for an exposure period of 1 hour,

and at site 3 for an exposure period of four hours. All sites were scored for erythema and oedema at 1, 24 and 72 hours following patch removal, and furthermore on day 7 and 14. The results are summarised in the Table below. Human data for skin irritation/corrosion is not available.

Table. Summary of the results of the skin irritation study

		1 hour exposure		4 hour exposure	
	Time	**Erythema**	**Oedema**	**Erythema**	**Oedema**
Draize score (average, scale 0 – 4)	**60 min**	1.0	3.0	2.0	3.0
	24 h	1.0	1.33	1.33	2.0
	48 h	0.67	1.67	1.33	2.0
	72 h	0.67	1.33	1.33	1.66
	7 days	3.0	1.0	4.0	2.0
	14 days	3.0	0.0	4.0	0.66
	Further details from day 14	–		Eschar in 1/3, necrosis in 1/3, flaking skin in 3/3	
Reversibility		No	Yes	No	No

Comments received during public consultation

The manufacturer agreed with the classification Skin Corr. 1B; H314.

One MSCA commented that the criteria for the sub-category 1B may not be met, as corrosive responses occurred only after 4-hour exposure, and therefore the sub-category 1C would be more justified. The DS replied that after re-evaluation, they agree that category 1C could be more appropriate.

Assessment and comparison with the classification criteria

Based on one good quality study, MBIT has clear skin corrosive properties. After 1-hour exposure, clear oedema was observed at 60 minutes, and clear erythema at 7 and 14 days after the exposure. After 4-hour exposure, both oedema and erythema were observed until 14 days post-exposure. The erythema was scored as severe both at 7 and 14 days post-exposure. Furthermore, after the 4-hour exposure and at 14 days, one animal had eschar, one exhibited necrosis and all three had flaking skin.

According to CLP criteria, a substance is a skin corrosive if it produces irreversible damage to the skin (namely, visible necrosis through the epidermis and into the dermis in at least one tested animal after an exposure duration of up to 4-hours). MBIT fulfils this criteria, as it produced necrosis (observed at 14 days) in one animal following the 4-hour exposure.

Category 1 further includes three subcategories. The criteria for sub-category 1A applies when destruction of skin tissue following exposure up to 3 minutes and observed within 1 h; sub-category 1B assumes responses following exposure ranging from 3 min to 1 h, observed within 14 days; sub-category 1C assumes responses that occur after exposures of 1-4 h and observed within 14 days. Therefore, based on the result of the study where corrosivity was observed between 1 to 4h of exposure with irreversible effects up to 14 days, RAC concludes that classification as **Skin Corr. 1C; H314; Causes severe skin burns and eye damage,** is warranted for MBIT instead of Skin Corr. 1B as proposed by the DS.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify MBIT as Eye Dam. 1; H318, based on the Skin Corr. 1 classification. An acute eye irritation study has not been performed with MBIT due to the corrosive properties observed in the acute dermal irritation study.

Comments received during public consultation

The manufacturer was in agreement with the proposed classification. Also one MSCA supported the classification.

Assessment and comparison with the classification criteria

According to the CLP, skin corrosive substances shall be considered as leading to serious damage to the eyes. RAC agrees that based on the skin corrosion study, classification as **Eye Dam. 1; H318; Causes serious eye damage,** is justified. The hazard statement H318 will not be added in the labelling column. Indeed, where a chemical is classified as skin corrosion, labelling for serious eye damage/eye irritation can be omitted as this information is already included in the hazard statement for skin corrosion (see section above) (European Commission, 2016).

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS did not evaluate respiratory sensitisation, as there was no relevant data available.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concludes that MBIT **cannot be classified for respiratory sensitisation due to lack of data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed to classify MBIT as Skin Sens. 1A; H317 – May cause an allergic reaction, based on three key studies: two local lymph node assay (LLNA) studies and one Buehler test study.

Both LLNA studies were performed in the mouse according to the OECD TG 429 without deviations, and were therefore considered to be reliable without restrictions. The resulting EC_3 values in these two studies were ≥ 10 455 ppm (1.04 %; 261 µg/cm^2) and 6 900 ppm (0.69 %; 173 µg/cm^2). The stimulation indexes in these two studies are shown in the Table below.

Table. Stimulation indexes in the two LLNA studies.

Study	Measured dose (ppm)	Stimulation index (test/control ratio)	Result
Study 1, 2008	0	1.0	Negative
	3 000	2.0	Negative
	10 000	2.9	Negative
	30 000	7.3	Positive
	100 000	10.4	Positive
	300 000	15.7	Positive
Study 2, 2009	0	1.0	Negative
	100	1.1	Negative
	3 000	2.6	Negative
	10 000	3.3	Positive
	30 000	7.5	Positive

The Buehler test was performed in the guinea pig in compliance with OECD TG 406 without deviations, and likewise considered reliable without restrictions. In this study, MBIT was tested at concentration of 600, 1 200 and 1 800 ppm, and reported to be a sensitiser at 1 800 ppm (0.18 %; 229 µg/cm^2), as 2/10 of the animals showed signs of allergic reactions at 24 h after the challenge.

In addition to the three key studies, as additional supporting information, the DS's proposal included one human repeated insult patch test (HRIPT) study (Davies *et al*., 1975), unfortunately several details describing how the study was performed are lacking. The study included 45 healthy volunteers, who received patches every 2 days for five weeks in the induction phase, for a total of 15 applications. A challenge application was applied 2 weeks later to each volunteer, and for the volunteers that showed evidence of possible sensitivity or atypical reactions, a second challenge application was applied 8 or 12 weeks later. After the first challenge application, 16/45 (36 %) of the volunteers had reactions that persisted, were atypical or greater than during the induction period. After the second challenge, it is reported that at 500 ppm, MBIT induced marked dermal reactions in 7 volunteers and mild skin responses in 2. The DS therefore concluded that at 500 ppm, dermal sensitisation was seen in 9/45 volunteers (20 %), which supports the results from the animal tests.

The DS did not propose an SCL, as by directly comparing the CLP criteria with the results of the LLNA and Buehler's test results, the potency classification for MBIT would be "strong", and therefore the GCL would be warranted. After the public consultation, the DS stated that a lower GCL for MBIT could be considered by RAC.

Comments received during public consultation

Five comments were received concerning skin sensitisation. The manufacturer agreed with the proposed classification Skin Sens. 1A. One comment was from a trade association. The commenter did not argue against the proposed classification, but against the setting of an SCL and the socio-economic issues it would pose for the industry.

Three comments were received from MSCAs, all were in agreement with the proposed classification Skin Sens. 1A. On the matter of setting an SCL, one MSCA was in agreement with the proposed GCL of 0.1 %. One MSCA noted that, while the animal data does not support setting of a concentration limit of 0.001 % for extreme sensitiser, the high number of human volunteers (9/45) sensitised in the HRIPT study may justify the setting of an SCL. One MSCA was of the opinion that an SCL is necessary due to a steep increase in frequency of contact allergy by isothiazolinone compounds. Furthermore, they pointed out that MBIT is structurally closely related to 1,2-benzothiazil-3-one (BIT), and also methylisothiazolinone (MIT) and methylchloroisothiazolinone (CMIT). Therefore, it is reasonable to assume that if MBIT would show cross-reactivity to the other isothiazolinones, it would contribute significantly to the broad

outcome of isothiazolinone allergy. Furthermore, they questioned why an SCL would be applied to BIT but not to MBIT, as LLNA data (included in the Annex 2, as well as in Table below) show MBIT to be a more potent sensitiser. Moreover, the sensitising capacity of MBIT is comparable to MIT, which was likewise considered based on LLNA data a "strong" sensitiser, but nevertheless an SCL was considered justified. Thus, as SCLs below the GCL were set for BIT, MIT and CMIT, they propose a lower SCL of 15 ppm also for MBIT.

Assessment and comparison with the classification criteria

The CLP criteria for Skin Sens. 1A are fulfilled, if the EC_3 value from an LLNA test is ≤ 2 %, or if in the Buehler test ≥ 15 % of the animals respond positively at ≤ 0.2 % topical induction dose. Both of these criteria are fulfilled by MBIT. In the two LLNA studies, the stimulation index was ≥ 3 (EC_3) at concentrations of 1.04 % and 0.69 %. In the Buehler test study, 20 % of the animals gave a positive result at 0.18 % MBIT. The result of the HRIPT test in human volunteers (20 % of the volunteers were reported to have been sensitised by MBIT) gives further evidence as to the skin sensitising properties of MBIT. Therefore, RAC agrees with the DS that classification as Skin Sens. 1A is justified.

RAC agrees with the two MSCAs that commented that an SCL is necessary. While there is no clinical information about MBIT, as it has not apparently been widely used as yet, it has been shown to exhibit strong skin sensitising properties in laboratory animals. The HRIPT study result further supports the view that MBIT is a potent skin sensitiser in humans.

Based on the two LLNA tests and one HRIPT study, it appears that MBIT may be as potent as MIT, as shown in the Table below. In the LLNA assay, MBIT induced clear reactions, and the dose levels were close to that of MIT, for which there are indications that skin sensitisation in humans may occur already at 100 or 50 ppm, or even at lower concentrations, and therefore an SCL of 15 ppm has been assigned. Furthermore, the EC_3 values for MBIT were notably lower than those for BIT, which has a concentration limit of 0.05 % (500 ppm). This SCL may not be fully protective for BIT, either, since there are some reports suggesting BIT caused skin allergies from PVC gloves containing 20-30 ppm of BIT (Aalto-Korte *et al*., 2006; 2007). Also the Scientific Committee on Consumer Safety (SCCS, 2012) concluded in their opinion that BIT is known to be a sensitiser in man and has induced sensitisation at circa 20 ppm in gloves.

Table. Comparison of MBIT, BIT, MIT and CMIT LLNA and HRIPT data (non-exhaustive), classification and concentration limits for skin sensitisation

	MBIT	**BIT (CAS 2634-33-5)**	**MIT (CAS 2682-20-4)**	**CMIT/MIT (3:1) (CAS 55965-84-9)**
LLNA result[a]	(1) EC_3 = 1.04 % (2) EC_3 = 0.69 % -> Skin Sens. 1A, strong	(1) EC_3 = 2.3 % (2) EC_3 = 32.4 % (2) EC_3 = 4.8 % (3) EC_3 = 10.4 % -> Skin Sens. 1(B, moderate; classified under DSD)	(1) EC_3 = 0.86 % -> Skin Sens. 1A, strong	(1) EC_3 = 0.003 % (2) EC_3 = 0.007 % -> Skin Sens. 1A
HRIPT result[b]	(3) 9/45 (20 %) volunteers showed dermal sensitization at 500 ppm (details lacking).	(3) 5/58 (9 %) at 725 ppm aq., 0/54 (0 %) at 360 ppm aq (details lacking).	1/116 (0.9 %) volunteers at 400 ppm and 1/210 (0.5 %) at 500 ppm. (No dose-response.)	–
SCL	This proposal: 0.0015 %	0.05 %	0.0015 %	0.0015 %
Studies	(1) & (2): see the Tbale above and the Background Document (3) Davies *et al.*, 1975	(1) Gerberick *et al.,* 2005 in Contact Dermatitis 16(4):157-202. (2) Botham *et al.*, 1991 in Contact Dermatitis 25(3):172-177 (3) Basketter *et al.*, 1999 in Contact dermatitis 40(3):150-154	(1): see RAC opinion for 2-methylisothiazol-3(2*H*)-one	(1) & (2): see RAC opinion for Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC No 220-239-6] (3:1)

[a] The LLNA data for MIT and CMIT/MIT (3:1) originates from their respective RAC opinions proposing harmonised classification and labelling at EU level. For BIT, the LLNA data originates from the public NICEATM LLNA databank.
[b] The HRIPT results are considered as supporting additional evidence, due to their non-validated nature and lack of available details.

Several isothiazolinones have furthermore been reported to share cross-reactivity for skin sensitisation (Schwensen *et al*., 2017; Aalto-Korte & Suuronen, 2017). Therefore, it is likely that MBIT will cross-react as well. A substantial amount of workers and consumers have already been sensitised to MIT and therefore, in addition to prevent further skin reactions by MBIT, it would be crucial to inform workers of the elicitation hazard with MBIT, which setting an SCL would allow.

In summary, RAC is of the opinion that an SCL is necessary for MBIT. For deriving the limit, starting with the exposure level used in the HRIPT test (500 ppm) is considered justified. However, 20 % of the volunteers were reported to become sensitised at this dose level, and therefore RAC is of the opinion that the SCL should be considerably lower than that, clearly less than a tenth of 500 ppm. Furthermore, there are published reports suggesting that a closely related isothiazolinone, BIT, may induce skin sensitisation in humans at already levels of 20–30 ppm in gloves.

Overall, RAC concludes that MBIT should be classified as **Skin. Sens. 1A; H334; May cause allergy or asthma symptoms or breathing difficulties if inhaled,** with an **SCL of 15 ppm (0.0015 %)**.

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

Summary of the Dossier Submitter's proposal

The DS proposed to not classify MBIT for STOT RE, due to conclusive data not sufficient for classification.

Repeated dose toxicity via oral route has been assessed both in rats and in dogs. In rats, 14 days range-finding study by administration of MBIT daily via the drinking water at the doses of 250, 500, 1 000 and 2 000 ppm (corresponding doses up to 83 mg/kg bw/d in males and up to 109 mg/kg bw/d in females) showed lower body weight gains and/or body weight losses, lower food and water consumption and clinical observations consistent with dehydration and poor nutrition (dermal atonia, thinness, decreased defecation and small faeces) mainly at the two highest dose levels. All 2 000 ppm group males and females were euthanized on study day 11 due to body weight losses, low food and water consumption and poor general condition. There were no test substance related macroscopic findings at the scheduled necropsies or in the male rat (group 4, 1 000 ppm) that was found dead on study day 9.

90-days drinking water study in rats was conducted at the doses of 50, 200 and 800 ppm (corresponding doses up to 50 mg/kg bw in males and up to 60 mg/kg bw in females). The study was performed according to the OECD TG and in compliance with GLP, and assessed as reliable without restrictions by the DS. There were no test substance related clinical or macroscopic findings. Also functional observational battery, locomotor activity, coagulation and ophthalmology parameters were unaffected by test substance administration. Effects observed by histopathology included local lesions (minimal to mild inflammation and oedema and some focal erosions) in forestomach of some high dose (60 mg/kg bw/d) females, which can be explained by the corrosive nature of the substance. In clinical chemistry, RBC counts were 6.2 % lower in the high dose group than in controls and reticulocyte counts were 31.6 % higher than in controls. Related to reticulocytosis, MCV was also increased. In clinical chemistry, total protein and globulin levels were statistically significantly decreased and A/G ratio increased in 200 and 800 ppm males. Urea nitrogen and phosphorus levels were statistically significantly increased at 800 ppm females. These effects were considered secondary to poor nutrition/hydration status. Mean water consumption was lower in all treated male groups and in 200 and 800 ppm female groups. Statistically significant decreases were seen in mean food consumption, mean body weights and body weight gains in 200 and 800 ppm group males, and in mean body weights and body weight gains in females at 800 ppm. Overall, DS concluded that observed findings in rats were mainly related to either local irritancy or poor palatability of the material.

Similarly in a 28 d study in dog, the main effects observed were lower food consumption and lower body weight gain and/or body weight losses in the two highest dose groups (4 000 and 8 000 ppm). In these dose groups, the dose level was lowered after the first week of exposure due to poor food consumption and weight losses to 1 000 ppm in the second highest group, and to 3 000 (after 1 week) - 500 (after second week) – 2 500 (after third week) ppm in the 8 000 ppm group. One group received a diet containing 2 000 ppm of MBIT for the whole 4 week period. In addition to low food consumption and related weight losses, no other clinical signs of toxicity or substance related mortality were observed. Potentially substance related decrease in white blood cells and reticulocyte counts were observed in the high group males (dosed with 8 000-3 000-500-2 500 ppm) after 4 weeks (but not yet after 3 weeks) of exposure.

Full 90-day study in dogs was conducted at the doses of 250, 750 and 2 000 ppm in diet (corresponding doses up to 59 mg/kg bw/d in males and up to 67 mg/kg bw/d in females). The study was performed in compliance with GLP, and assessed as reliable without restrictions by the DS. Clinical observations included thinness and/or dermal atonia, lower body weight gains (or body weight losses), and lower food consumption in 2 000 ppm group males and females. No test material related mortality occurred. In clinical chemistry, only effects observed were lower absolute lymphocyte counts in the highest group females. Relative liver weights of the high dose group were higher than that of controls both in males and in females and absolute and relative adrenal weights were higher in high dose females than in controls. Only histopathological effects observed were, however, hypertrophy of the mucus-secreting cells of the surface epithelium in the stomach of 750 ppm and 2 000 ppm animals and thymic atrophy/involution in animals

exposed to 2 000 ppm. Thymic effects were considered stress related and effects in stomach were considered to reflect irritant effect of MBIT on the gastric mucosa. None of the observed effects were considered relevant for classification.

Repeated dose toxicity via the inhalation route was assessed in one subchronic 90 day study in rats. The study was performed according to the OECD TG 413 (nose-only) and in compliance with GLP, and assessed as reliable without restrictions by the DS. The analytically determined concentrations were 0, 0.04, 0.19, 0.75 and 7.04 mg MBIT/m^3, 10 males and 10 females were included at each dose level. The solvent was water, MBIT delivered as a liquid aerosol, and the particle size was targeted to have an average mass median aerodynamic diameter < 3 µm.

No treatment-related clinical signs of toxicity were observed in the study, and no treatment-related mortality occurred. Mean body weight gains were decreased in the females exposed to 7.04 mg MBIT/m^3 throughout the study in a statistically significant manner. Also for males in the high dose group, the mean body weight gains were decreased throughout the study, but the decrease was statistically significant only on test days 47 and 54. Likewise, mean food consumption was statistically significantly decreased in the females of the high dose group throughout the study, and for the males of the high dose group during the first three weeks of the study. Furthermore in the high dose group, the terminal fasting body weights of females were statistically significantly decreased (-14.8 %). There was a decrease also in the fasting terminal weights of males (-10.3 %), the decrease was not statistically significant. The treatment-related reduction in body weight gain, feed consumption and terminal body weights of both sexes in the high dose group were reported to likely be due to the irritant effects of repeated inhalation exposure.

Localised irritant effects of the test material were observed at the portal of entry, starting at the dose of 0.75 mg/m^3. Furthermore, in males and females exposed to 0.75 or 7.04 mg/m^3, treatment-related histopathological changes were reported in the anterior nasal cavity and anterior larynx, consistent with localised portal of entry irritant effects of the test material at the point of contact with the upper respiratory tract. The changes included very slight or slight hyperplasia and hypertrophy of mucous cells, very slight, slight or moderate squamous metaplasia, very slight or slight subacute to chronic inflammation of the mucosa, very slight fibrosis within the lamina propria of the mucosa, and very slight multifocal haemorrhages and the presence of small numbers of pigment-laden macrophages of the mucosa.

In the males of the high dose group, mean absolute lung weights were significantly decreased (28.6 %), and mean relative testes weights were significantly increased. In females, mean absolute liver and ovary weights were significantly decreased (15 % and 21.6 %, respectively) in the high dose group. Furthermore in females, mean relative kidney and brain weights were significantly increased (12 % and 15 %, respectively) in the high dose group. All of these changes were interpreted to be secondary to the decreases in body weight, as they were not associated with any treatment-related histopathological alterations. There was also no histopathological evidence of any primary systemic toxicity.

There were no treatment-related ophthalmoscopic findings, nor statistically significant or treatment-related changes in any of the haematological parameters, clinical chemistry parameters or urine analysis of either sex at any dose level.

Overall, based on these five studies DS concluded that no classification for STOT RE is warranted.

Comments received during public consultation

No comments were received regarding STOT RE.

Assessment and comparison with the classification criteria

According to CLP regulation, substances are classified for target organ toxicity STOT RE 1 if they have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

According to the criteria, classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose toxicity studies conducted in experimental animals are seen at generally low exposure concentrations. Guidance values for different routes are given to be used as part of the weight of evidence approach and to assist with decisions about classification.

Substances are classified in Category 2 for target organ toxicity (repeat exposure) (STOT RE 2) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. On the basis of evidence from studies in experimental animals it can be presumed that the substance has the potential to be harmful to human health following repeated exposure.

In the case of MBIT, specific histopathological effects observed in both rats and dogs included local effects in forestomach or in gastric mucosa. In dogs, hypertrophy of the mucus-secreting cells of the surface epithelium in the stomach was seen at 750 ppm (26-27 mg/kg bw/d) and 2 000 ppm (59-67 mg/kg bw/d) in diet. In rats, there were minimal to mild inflammation and oedema and some focal erosions) in forestomach of some high dose (60 mg/kg bw/d) females. After inhalation, there were very slight or slight hyperplasia and hypertrophy of mucous cells, very slight, slight or moderate squamous metaplasia, very slight or slight subacute to chronic inflammation of the mucosa, very slight fibrosis within the lamina propria of the mucosa, and very slight multifocal haemorrhages and the presence of small numbers of pigment-laden macrophages of the mucosa starting at 0.75 mg/m^3. These effects fit with the corrosive nature of the substance. Substance has been already classified as corrosive, and respiratory irritation has been also specifically addressed by STOT SE 3 classification. Thus, no STOT RE classification is warranted for these irritant effects.

In haematology/clinical chemistry, some effects were observed both in rats and dogs. In rats after oral exposure, RBC counts were 6.2 % lower in the high dose group (50-60 mg/kg bw/d) and reticulocytes were 31.6 % higher than in controls. MCV was increased due to reticulocytosis. In 4-week dog study, reticulocyte counts were lower when compared to the controls in males after week 4, but not after week 3 in high dose animals (in controls reticulocyte mean percentage was 1.2 % and in high dose males it was 0.5 %). White cell counts in high dose males were 9.07 vs 17.06 thousands/µL in controls (on the other hand, in controls after 3 week white cell count was 12.7). However, in 90-day oral study in dogs, only findings in haematology were lower absolute lymphocyte counts in the high dose (2 000 ppm, 67 mg/kg bw/d) females. Thus, these changes in haematological parameters were not very consistent and clear dose-response was not observed. In addition, some of the changes, like 6.2 % decrease in RBC count, are considered very minimal. In inhalation study in rats, no haematological effects were observed. Overall, these effects are not considered relevant for STOT RE classification.

In the rat 90 d oral study, total protein and globulin levels were slightly, but statistically significantly decreased and A/G ratio was increased in 200 and 800 ppm males. At these dose levels lower food consumption was observed in 800 ppm males but also in 200 ppm males at many time points. Urea nitrogen and phosphorus levels were slightly (but statistically significantly) increased at 800 ppm in females. This may be related to the reduced water consumption observed in these females. No clinical chemistry effects were observed in dogs. Since effects in clinical chemistry were only very mild, non-consistent across the studies and sexes and were

likely to be related to the poor nutritional/hydration status of the animals, these effects are not considered relevant for classification.

Lower body weights were observed at highest dose level in all repeated dose studies and were accompanied with lower food and/or water consumption.

Overall, RAC concurs with DS that **no classification for STOT RE** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed to not classify MBIT for germ cell mutagenicity, as the data was conclusive but not sufficient for classification. The dossier includes three *in vitro* and one *in vivo* mutagenicity assays, all of which were considered reliable without restrictions, summarised in Table below.

Table. Summary of the mutagenicity studies

Method	Method	Test system	Results	Conclusion
In vitro gene mutation study in bacteria	OECD TG 471, ± metabolic activation (S9)	*S. Typhimurium* (TA 1535, 1537, 98, 100) *E. coli* (WP2 uvrA)	No positive mutagenic responses in the two independent studies (initial & confirmatory) performed. Cytotoxicity observed at high doses [(50-) 150-5 000 µg/plate].	Negative
In vitro mammalian chromosome aberration test	OECD TG 473, ± metabolic activation (S9)	Human peripheral blood lymphocytes (HPBL)	No statistically significant increase in the number of cells with structural or numerical aberrations.	Negative
In vitro mammalian cell gene mutation test	OECD TG 476, ± metabolic activation (S9)	Chinese hamster ovary cells (CHO-K_1)	No increases in mutant frequencies in two independent tests (initial & confirmatory) performed. Cytotoxicity at higher concentrations (≥ 4.0-5.0 µg/mL without S9 and ≥ 8.0-10 µg/mL with S9 mix)	Negative
In vivo Mammalian erythrocyte micronucleus test	OECD TG 474	Mouse (ICR)	No significant increase in the incidence of micronucleated polychromatic erythrocytes. Mortality was observed in the high dose group (200 mg/kg bw).	Negative

Comments received during public consultation

No comments were received concerning germ cell mutagenicity.

Assessment and comparison with the classification criteria

Substances are classified for mutagenicity in Cat. 1, if they are known to induce heritable mutations or are regarded as if they induce heritable mutations in the germ cells of humans. Cat. 2 applies to substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans, based on positive evidence obtained from *in vivo* experiments in mammals and/or in some cases from *in vitro* experiments.

The dossier includes four studies on mutagenicity, three of which were performed *in vitro* and one *in vivo*. All of the studies were conducted according to OECD TG and have been evaluated by the DS as reliable. The result was negative in each of the studies. Therefore, RAC agrees with the DS that **classification for germ cell mutagenicity is not warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS did not propose a classification for carcinogenicity, as there is no data available on carcinogenicity or chronic toxicity of MBIT. However, the DS presented several arguments to lessen concern of carcinogenicity. MBIT has been tested in several assays with repeat dose protocols, including 90 day studies in the rat and in the dog. In addition, several studies have been performed to assess reproductive toxicity. There are also negative data on genotoxicity from one *in vivo* and three *in vitro* studies, arguing against a potential genotoxic mechanism of carcinogenesis.

Irrespective of the species or the route of administration, the major toxicity observed by MBIT in the repeated dose studies is irritation/corrosion at the site of primary contact, and clinical signs of toxicity or mortality observed have been judged secondary to these irritant effects. There were no histopathological effects observed in any tissues distant from the site of dosing in any of the studies. Furthermore, there was no evidence suggestive of a potential endocrine mechanism of carcinogenesis.

In addition, the ADME properties of MBIT have been studied. After single or repeated oral exposures, MBIT appears to be extensively metabolised in the rat (both sexes), much like other isothiazolinones. Non-metabolised MBIT was not found in urine or faeces. The findings do not indicate that MBIT would bioaccumulate in rat tissues, as most of the dosed radioactivity was recovered within 24 h of exposure in excreta (total mean recoveries > 97 %).

The DS concluded that given the lack of significant organ toxicity, genotoxic potential and endocrine activity, MBIT is unlikely to demonstrate carcinogenic potential.

Furthermore, structurally related isothiazolinones, CMIT/MIT (3:1 mixture) and OIT have been extensively studied in repeated dose studies, including three chronic studies. They have demonstrated point of contact irritation and/or corrosion, but no significant systemic toxicity or evidence of a possible endocrine mechanism of carcinogenicity. They have shown some genotoxicity *in vitro*, but have been reported to not be genotoxic *in vivo*. Furthermore, two carcinogenicity studies conducted on CMIT/MIT and one in OIT have not indicated carcinogenic potential.

Comments received during public consultation

No comment were received during public consultation regarding carcinogenicity.

Assessment and comparison with the classification criteria

A substance is classified in Cat. 1A or 1B for carcinogenicity on the basis of epidemiological and/or animal data, if it is known or presumed to have carcinogenic potential for humans. Category 2 applies for suspected human carcinogens on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. For MBIT, there is no data on carcinogenicity nor indications of carcinogenic potential, therefore **no classification for carcinogenicity** is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification for fertility or developmental effects. This conclusion is based on a two generation reproduction toxicity study in rat (OECD TG 416) and developmental toxicity studies in rats and rabbits.

Fertility

In the two generation study, rats were exposed to MBIT via drinking water at the doses of 50, 200 and 800 ppm. Due to excessive toxicity noted in the 800 ppm group, this exposure level was reduced to 400 ppm (F0 male study week 16 or F0 female lactation days/F1 pup postnatal days [PND] 15-20) and maintained at this exposure level throughout the remainder of the F0 generation and for the entire F1 generation. The corresponding dose levels were: 3-11 mg/kg bw/d (50 ppm), 11-45 mg/kg bw (200 ppm) and 23-83 mg/kg bw (400 ppm).

No test material related mortality was observed in parental animals. Mean water consumption was reduced in a dose-related manner both in male and female F0 animals. Mean body weight gain was reduced in F0 males throughout exposure at 800 ppm, but following the reduction in the exposure level mean body weight gain was slightly higher than the control group values. In F0 females, body weight gain was only affected during the first week of exposure. Reduced food consumption was observed both in F0 males and females during the whole study at the highest dose. Test substance related clinical observations included increased incidences of red and yellow material on various body surfaces in the 800/400 ppm group primarily during the period of exposure to 800 ppm. Additionally, several females in this group were noted with an unkempt appearance during lactation days 15-20. No effects on the mating, gestation, number of F1 pups born, pup sex ratio and pup survival during the pre-weaning period were noted. F1 pup body weights were lower both in females and males during PND 4-28 in the highest (400/800 ppm) dose group. F1 female pups in the highest dose group showed uneven hair growth, striped hair growth and unkempt appearance at PND 8, 14 and/or 21. No clinical signs were observed in males. A delay in the mean age of attainment of vaginal patency and balanopreputial separation were noted for F1 pups in this group. This was accompanied with a lower mean body weight when compared to the controls. Sperm parameters were unaffected both in F0 and F1 males and no treatment-related microscopic changes were observed in any reproductive organs of the F0 and F1 male and female rats at any exposure level. A low incidence of focal papillary oedema was observed in F0 males and females in the 800/400 ppm and in F1 males in the 400 ppm group. This was associated with an increased incidence and severity of focal cortical and medullary tubular nephropathy in the F0 males in this dose group.

In F1 generation mean water consumption was reduced in 400 ppm group. Food consumption was statistically significantly reduced in males but not in females. Body weights of F1 males for the whole generation were 23 % lower than in controls. Body weights of pre-mating F1 females were 14.3 % lower than the body weight of controls. No effects on the mating, gestation, number of F2 pups born, pup sex ratio and pup survival during the pre-weaning period were noted. No clinical findings were noted in F2 pups. Test substance related lower mean F2 pup body weights were observed both in male and female pups at 200 ppm (8.4 % for males, 16.9 % for females) and 400 ppm (9.0 % for males and 18 % for females) on PND 21 when compared to the controls. This was related to the lower body weight gain during PND 7-14 and/or PND 14-21.

Since no effects in fertility were observed in this study, DS concluded that no classification for fertility is warranted.

Developmental toxicity

Developmental toxicity study in rats was conducted by gavage at dose levels of 2, 5 and 18 mg/kg bw/d. In the 18 mg/kg bw/d group, 3/25 females were euthanized in extremis on gestation day 9 or 11 due to body weight losses, reduced food consumption, and poor clinical condition. Observed clinical findings (rales, gasping, limbs and/or body cool to touch, red material on the nose, mouth, and/or forelimbs, and/or yellow material on the anogenital or urogenital areas) were attributed to the locally irritating nature of the test substance. There were no test substance related clinical findings noted in the 2 and 5 mg/kg bw/d groups. Maternal mean body weight in the 18 mg/kg bw/d group was 11.6 % lower compared to the controls whereas in 2 and 5 mg/kg bw/d groups no difference to the controls were observed.

Mean foetal weights in the 18 mg/kg bw/d group were 7.9 % (male) and 10.5 % (female) lower than in the concurrent control group values. Increased incidence of reduced ossification was reported in the 18 mg/kg bw/d group showing also clinical signs of maternal toxicity and reduced maternal weight gain. No malformations or other variations were observed at any dose level.

Developmental toxicity study in rabbits was conducted by gavage at dose levels of 2, 5 and 20 mg/kg bw. In the 20 mg/kg bw/d group, 1/25 females were euthanized in extremis on gestation day 21 due to body weight loss and minimal food consumption.

There were no test substance related clinical findings noted in the 2 and 5 mg/kg bw/d groups. Maternal mean body weight in the 18 mg/kg bw/d group was 11.6 % lower compared to the controls whereas in 2 and 5 mg/kg bw/d groups no difference to the controls were observed. Test substance related decreased defecation was noted for all females in the 20 mg/kg bw/d group generally during gestation days 9 through 29 in parallel with decreased food consumption. In this group, mean body weight was 8.7% lower than the control group during the gestation. Reduced body weight gain was mainly due to body weight losses observed during the first week of gestation.

Intrauterine growth and survival were unaffected by the test substance administration at all dosage levels. Only findings were higher mean litter proportions of 13th full rib(s) and 27 pre-sacral vertebrae noted in the 20 mg/kg bw/d group, which are common skeletal variations among rabbits and were not considered as adverse. Based on the lack of any relevant developmental toxicity findings DS concluded that no classification for developmental toxicity is warranted.

Comments received during public consultation

There were no specific comments on reproductive toxicity.

Assessment and comparison with the classification criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

Fertility

In the case of MBIT, only animal data on reproductive effects is available. Regarding effects on fertility, a two generation reproductive toxicity study did not show any effects on fertility parameters; only effects observed were slight reductions in post-natal body weight gain in F1 generation at the highest dose (400 ppm) and in F2 generation at the mid (200 ppm) and highest dose (400 ppm). Lower body weights were also observed at the highest dose in F0 and F1 animals and were usually accompanied with lower food and/or water consumption. RAC agrees with DS that the study gave no indications on fertility effects and **no classification for fertility is warranted**.

Development

For effects on development, MBIT has been studied in two developmental toxicity studies in rats and in rabbits. In rats, highest dose level (18 mg/kg bw/d) resulted in clinical signs of toxicity and reduced body weight gain of the dams. Three out of 25 females were euthanized due to their poor condition. The only effect observed in foetuses were reduced mean foetal weight at the highest dose. Also, increased incidence of reduced ossification was reported but no incidences were given in CLH report. According to the data, the percentage of skeletal variations was 39.4, 29.7, 31.6 and 43.9% for 0, 2, 5, 18 mg/kg bw/d, respectively. RAC considers the slight effects seen at the highest dose secondary to the maternal toxicity observed at this dose level.

In rabbits, the highest dose, 20 mg/kg bw/d, resulted in reduced maternal weight gain (according to the data given in IUCLID file, body weight changes during GD 7-29 (mean ± SD) for 0, 2, 5, 20 mg/kg bw/d were 145 ± 215.3, 199 ± 175.9, 211 ± 165.3, 37 ± 148.4, respectively). Reduced weight gain was mainly due to body weight losses during the first week of gestation. Food consumption was statistically significantly reduced and also reduced defecation was observed at the high dose. No effects on foetal survival, intrauterine growth or incidence of malformations or visceral variations were observed at any dose level. Incidence of 13^{th} full rib was increased in the high dose group (incidences in 0, 2, 5 and 20 mg/kg bw/d were 42, 79, 72 and 105, respectively) as well as incidences of 27^{th} pre-sacral vertebrae (incidences in 0, 2, 5 and 20 mg/kg bw/d were 16, 25, 23, 34, respectively). On the other hand, incidence of rudimentary 13^{th} rib was lower in high dose group when compared to the controls (incidences in 0, 2, 5 and 20 mg/kg bw/d 22, 30, 27 and 11). RAC concurs with the DS that these variations are very common in New Zealand White rabbits and in the absence of any other developmental toxic findings they are considered as minimal adversity. In addition, it is noted that these findings were observed only at the highest dose which resulted in reduced food consumption, reduced defecation (which in rabbits may result in malnutrition) and reduced body weight gain. Therefore, RAC does not consider these effects as specific indications of teratogenic potential of MBIT. Since rat developmental toxicity study did not show specific developmental toxicity, either, RAC concurs with DS and proposes **no classification for developmental effects.**

There is no data on the excretion of MBIT or its metabolites to breast milk or on the adverse effects via lactation. Therefore, **no classification for effects via lactation** is proposed.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

No data were included in the Background Document.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No classification is proposed due to lack of data.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed that MBIT is rapidly degradable and that it should be classified as:

- Aquatic Acute 1; H400 (M-factor of 1) based on a 96-h LC_{50} of 0.24 mg/L for fish and a 48-h E_rC_{50} of 0.24 mg/L for algae.

- Aquatic Chronic 2; H411 based on a 48-h NOE_rC of 0.012 mg/L for algae.

Degradation

MBIT is hydrolytically stable over 5 days at 50 °C and pH 4, 7 and 9. This equates to a half-life at 25 °C of >1 year at pH 4, 7 and 9.

In an aqueous photolysis study at pH 7, MBIT underwent primary degradation, producing eleven degradants over 168 hours. The two main degradants detected at > 10 % of the applied amount of MBIT were 2-(methylcarbamoyl)-benzene sulfonic acid and 2-(carbamoyl)-benzene sulfonic acid (the other nine were present at 1.4 – 8.6 % of the applied amount of MBIT by the end of the study).

A GLP compliant ready biodegradation test according to OECD TG 301B (CO_2 Evolution Modified Sturm test) using ^{14}C-MBIT resulted in less than 1 % degradation after 28 days based on carbon dioxide evolution and Applied Radioactivity (AR). MBIT was not detected in the supernatant by day 28 and underwent rapid primary degradation (not mineralisation) over the study period. The primary degradation DT_{50} was 2.1 days. Two major degradants were detected in the test vessels (*N*-methyl 2-(methylthio)benzamide and hydroxy-2-methylsulfinyl-benzamide / *N*-methyl-2-(methylsulfinyl)benzamide, comprising ~ 75 % AR and ~ 25 % AR, respectively). Based on these results, MBIT is not readily biodegradable.

A 14d aerobic freshwater simulation test (OECD TG 309) indicates that the substance undergoes primary degradation but minimal mineralisation (< 1 % AR by day 14). Study half-lives for primary degradation at 20 ± 2 °C were 0.34 to 0.61 hours. At an environmentally relevant temperature of 12 °C, primary degradation half-lives would be 0.03 to 0.05 days. Major degradants are described as *N*-methyl-2-(methylthio)-benzamide, 2-(methylcarbamoyl)-benzene sulfonic acid and 2-carbamoyl-benzene sulfonic acid.

A sewage treatment simulation test is also available but this is not considered relevant for classification purposes due to potential micro-organism adaptation.

The DS provided additional data to demonstrate that the three main identified MBIT degradants are not classifiable (see Table below). Data for the other identified degradants were not provided.

Table: Fate and ecotoxicity data for MBIT degradants

Method	Result	Remarks	Reference
Degradant 1: *N*-methyl-2-(methylthio)-benzamide			
Water solubility (at 25 °C) QSAR estimate	2.28×10^3 mg/L		EPIWIN
Log K_{OW} (at 25 °C) QSAR estimate	1.80		EPIWIN
Ready biodegradation OECD TG 301F, GLP	5.4 % degradation	Not rapidly biodegradable	Chai and Hales, 2014
Ready biodegradation QSAR estimate	Not readily biodegradable		EPIWIN
Acute toxicity to fish (*Oncorhynchus mykiss*) OECD TG 203, GLP	LC_{50} > 101 mg/L		Currie *et al*., 2014a
Acute toxicity to fish QSAR estimate	96-h LC_{50} = 33.3 mg/L		EPIWIN
Acute toxicity to invertebrates (*Daphnia magna*) OECD TG 202, GLP	EC_{50} > 101 mg/L		Currie *et al*., 2014b
Acute toxicity to invertebrates QSAR estimate	48-h EC_{50} = 17.5 mg/L		EPIWIN
Algal growth inhibition (*Pseudokirchneriella subcapitata*) OECD TG 201, GLP	E_rC_{50} > 101 mg/L		Currie *et al*., 2014c
Algal growth inhibition QSAR estimate	96-h EC_{50} = 0.451 mg/L		EPIWIN
Degradant 2: 2-(methylcarbamoyl)-benzene sulfonic acid			
Water solubility (at 25 °C) QSAR estimate	1.0×10^6 mg/L		EPIWIN
Log K_{OW} (at 25 °C) QSAR estimate	-1.95		EPIWIN
Ready biodegradation OECD TG 301F, GLP	8.3 % degradation	Not rapidly biodegradable	Chai and Hales, 2014
Ready biodegradation QSAR estimate	Not readily biodegradable		EPIWIN
Acute toxicity to fish (*O. mykiss*) OECD TG 203, GLP	LC_{50} > 101 mg/L		Currie *et al*., 2014a
Acute toxicity to fish QSAR estimate	96-h LC_{50} > 1 000 mg/L		EPIWIN
Acute toxicity to invertebrates (*Daphnia magna*) OECD TG 202, GLP	EC_{50} > 101 mg/L		Currie *et al*., 2014b
Acute toxicity to invertebrates QSAR estimate	48-h EC_{50} > 1 000 mg/L		EPIWIN
Algal growth inhibition (*Pseudokirchneriella subcapitata*) OECD TG 201, GLP	E_rC_{50} > 101 mg/L		Currie *et al*., 2014c
Algal growth inhibition QSAR estimate	96-h EC_{50} = 59.8 mg/L		EPIWIN
Degradant 3: 2-carbomyl-benzene sulfonic acid			
Water solubility (at 25 °C) QSAR estimate	1.0×10^6 mg/L		EPIWIN
Log K_{OW} (at 25 °C)	-3.15		EPIWIN

Method	Result	Remarks	Reference
Degradant 1: *N*-methyl-2-(methylthio)-benzamide			
QSAR estimate			
Ready biodegradation QSAR estimate	Not readily biodegradable		EPIWIN
Acute toxicity to fish QSAR estimate	96-h LC_{50} = 3.09 × 10^6 mg/L		EPIWIN
Acute toxicity to invertebrates QSAR estimate	48-h EC_{50} = 2.94 × 10^5 mg/L		EPIWIN
Algal growth inhibition QSAR estimate	96-h EC_{50} = 20.7 mg/L		EPIWIN

Note: Although the comparison is not made in the Background Document (Annex I), the EPIWIN predictions appear to significantly over-estimate the level of acute toxicity for this type of substance.

In summary, the DS considered MBIT undergoes rapid primary degradation to degradants which are likely to be stable but unlikely to meet the hazard classification criteria. On this basis the DS considered MBIT to be rapidly degradable.

Bioaccumulation

The octanol-water partition coefficient (log K_{OW}) is < 1.6. No further information is available. This is below the CLP criterion for a bioaccumulative substance (log K_{OW} > 4), so the DS considered that MBIT does not have potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following Table (the key endpoints used in hazard classification are highlighted in bold). All study results are expressed in terms of mean measured concentrations, unless stated otherwise. The 95 % confidence intervals have been included in the Table below where relevant to give an indication of the variability of the data.

Table: Summary of relevant information on aquatic toxicity of MBIT

Method	Test organism	Endpoint	Toxicity values (mg/L)	Reference
Short-term toxicity to fish				
OECD TG 203 (flow-through)	*Oncorhynchus mykiss* (Rainbow Trout)	96-h LC_{50}	**0.24** (95 % CI: 0.11–0.46)	Sayers, 2007a
OECD TG 203 (flow-through)	*Cyprinodon variegatus* (Sheepshead Minnow)	96-h LC_{50}	1.5 (95 % CI: 1.0–2.2)	Soucy, 2009a
Long-term toxicity to fish				
OECD TG 210 (flow-through)	*Pimephales promelas* (Fathead Minnow)	32-d NOEC	0.16	Hamitou, 2009a
Short-term toxicity to aquatic invertebrates				
OECD TG 202 (flow-through)	*Daphnia magna*	48-h EC_{50}	0.92 (95 % CI: 0.65–1.3)	Sayers, 2007b
US EPA OPPTS 850.1035 (flow-through)	*Americamysis bahia* (mysid shrimp)	96-h LC_{50}	**0.48** (95 % CI: not provided)	Soucy, 2009b
Long-term toxicity to aquatic invertebrates				
OECD TG 211 (flow-through)	*Daphnia magna*	21-d $NOEC_{survival}$	0.42	Hamitou, 2009a
Toxicity to algae and aquatic macrophytes[a]				

Method	Test organism	Endpoint	Toxicity values (mg/L)	Reference
Short-term toxicity to fish				
OECD TG 201 (static)	*Pseudokirchneriella subcapitata*	24-h E_rC_{50} 48-h E_rC_{50} 72-h E_rC_{50}	0.419 (95 % CI: 23.6–27.8) **0.373** (95 % CI: 0.350–0.396) 0.319 (95 % CI: 0.264-0.374) (based on initial measured concentrations)	Hoberg, 2007 and subsequent evaluating Member State (eMS) reanalysis [b]
			<u>Reanalysis by eMS:</u> 0.474 (95 % CI: not provided) **0.361** (95 % CI: not provided) 0.315 (95 % CI: not provided) (based on initial measured concentrations)	
		24-h E_rC_{10} 48-h E_rC_{10} 72-h E_rC_{10}	0.334 (95 % CI: 0-22.6) **0.129** (95 % CI: 0.107–0.150) 0.167 (95 % CI: 0.079-0.253) (based on initial measured concentrations)	
			<u>Reanalysis by eMS:</u> 0.321 (95 % CI: not provided) **0.157** (95 % CI: not provided) 0.166 (95 % CI: not provided) (based on initial measured concentrations)	
		24-h NOE_rC 48-h NOE_rC 72-h NOE_rC	0.16 0.027 0.068 (based on initial measured concentrations)	
			<u>Reanalysis by eMS:</u> 0.16 0.027 0.068 (based on initial measured concentrations)	
		48-h E_rC_{50} 48-h E_rC_{10} 48-h NOE_rC	<u>Reanalysis by eMS using mean measured (time-weighted average) concentrations for Biocidal Products Regulation:</u> 0.24 (95 % CI: not provided) 0.09 (95 % CI: not provided) 0.012	
OECD TG 201 (static)	*Skeletonema costatum*		Study results included in CLH Annex but as the study validity criteria were not met, the study is not considered reliable and further details are not included.	Softcheck, 2009 and subsequent evaluating Member State reanalysis

Note:
a - Presented results from CLH report and Annex 1
b - Test guideline validity criteria were not met at 96 hours so 96-h endpoints are not included.
CI - confidence interval

Data for *Chironomus* species were reported but are not relevant for hazard classification as the substance was spiked in the sediment systems.

Comments received during public consultation

One MSCA and a manufacturer agreed with the proposed environmental hazard classification with no further comment. Two MSCAs provided specific comments, as follows.

Hazard labelling

One MSCA stated that labelling with H400 (Very toxic to aquatic life) alone is not appropriate as it does not communicate the long-term hazard. The DS agreed that labelling with H410 (Very toxic to aquatic life with long lasting effects) would cover both the short- and long-term hazards avoiding duplication (in accordance with Article 27 of the CLP Regulation).

Degradation

One MSCA considered that MBIT is not rapidly degradable as there was insufficient information to fully consider the classification of all MBIT degradants. A second MSCA asked whether all MBIT degradants had been identified and for further information about their chronic ecotoxicity. In response, the DS confirmed that all degradants detected in the fate studies were described in the CLH report and that these degradants would not be classified based on the ecotoxicity data in the CLH report. They also noted that in general MBIT degradants were less toxic than the parent substance. The DS drew attention to the similarities of 2-(methylcarbamoyl)-benzene–sulfonic acid and 2-carbamoyl-benzene–sulfonic acid (effectively concluding that they will have the same hazard classification). RAC agrees that the reported measured acute data suggest that the three main degradants are unlikely to be classifiable for environmental hazard. However, the ECHA Read Across Assessment Framework has not been followed to provide a transparent analysis, no additional degradant ecotoxicity data were provided, and RAC notes that full details of the studies and predicted data for the degradants are not available in the Background Document (Annex I) or response to comments. RAC therefore asked ECHA to perform a QSAR analysis for the degradants, which is summarised as supplemental information below. In addition, the DS provided summaries of the available ecotoxicity studies for the degradants at the request of the rapporteur (test substance concentrations were $\geq$ 80 % of initial concentrations during all of the tests).

Aquatic toxicity

One MSCA pointed out that the CLP Guidance (section 4.1.3.1.1) states that if available, EC_{10} endpoints should be used in preference to NOECs. In response, the DS stated that the NOE_rC endpoint was used for the aquatic PNEC in the biocides risk assessment as it was lower than the EC_{10} endpoint and that this was agreed by the Biocidal Products Committee (BPC) (BPC-WG IV/2016). RAC supports the use of the EC_{10}, since it is a statistically derived term, whereas the NOEC depends on the selected treatment concentrations (see Guidance on the Application of the CLP Criteria, referring to OECD (2006)). The same MSCA considered that the specific mode of action for isothiazolinones (whereby the substance is taken up by algal cells and transformed; it is this process which induces the toxic response) means that initial measured algal concentrations are more appropriate for hazard classification endpoints as they reflect the concentration which will induce the toxic effect in algae. In response, the DS stated that the biocide assessment was agreed based on mean measured endpoints for algae due to the significant losses of MBIT over the study period. RAC considers that use of mean measured concentrations provides an unrealistically conservative estimate of the concentration of test item required to induce the observed level of toxic response. In addition, varying losses are observed between low and high dose treatments since at higher doses, high algal inhibition results in lower losses because viable algal cells are not available to take up the test item after the initial toxic response. As test item loss is dependent on algal cell concentrations and differing kinetic losses would be observed across treatments, it is unclear how representative a dose-response curve based on time-weighted average concentrations would be for shorter test duration endpoints (i.e. 48 hours). RAC therefore considers that initial measured concentrations are more appropriate for hazard classification purposes.

Additional queries covered details of some of the studies. The DS confirmed that:

i) the Softcheck (2009) study on *Skeletonema costatum* is not reliable;

ii) 96-hour endpoints from the Hoberg (2007) algal study are not reliable, but test guideline validity criteria were met at 48 hours;
iii) chronic fish and *Daphnia* endpoints were based on mean measured concentrations; and
iv) the acute *Chironomus dilutus* study was conducted in compliance with a standard test guideline.

Assessment and comparison with the classification criteria

Degradation

MBIT is not readily biodegradable, but undergoes rapid primary degradation to several degradants that appear to be more stable. Experimental ecotoxicity data are available indicating that three of these degradants are unlikely to be classified as hazardous. However, based on QSAR analyses performed by ECHA, some of the degradants appear to be classifiable as hazardous (see supplemental analysis). RAC therefore considers that MBIT should be treated as not rapidly degradable for the purpose of hazard classification. This view could change if further reliable information on all of the degradants is provided in future.

Bioaccumulation

The substance is not potentially bioaccumulative because its log K_{OW} value (< 1.6) is below the CLP Regulation threshold of 4.

Acute Aquatic toxicity

Short-term aquatic toxicity data are available for three trophic levels. The lowest endpoints for fish and invertebrates are:
96-h LC_{50} of 0.24 mg/L for *Oncorhynchus mykiss*
96-h LC_{50} of 0.48 mg/L for *Americamysis bahia*

One reliable algal growth inhibition study is available (Hoberg, 2007). MBIT is an isothiozolinone with a specific mode of action whereby the substance is taken up by algal cells and transformed. It is this process which induces the toxic response. The 48-h E_rC_{50} is 0.361-0.373 mg/L (72-h E_rC_{50} values are also within the range 0.1 to 1 mg/L), based on initial measured concentrations.

As these endpoints are below 1 mg/L, the substance meets the criteria for **classification with Aquatic Acute 1, H400; Very toxic to aquatic life**. As 0.1 < L(E)C_{50} ≤ 1 mg/L, **the M-factor is 1**. Therefore RAC supports the DS's proposal.

Chronic Aquatic toxicity

Chronic ecotoxicity data are available for all three trophic levels. High algal sensitivity is expected in comparison with other isothiazolinones (MIT, CAS No 2682-20-4 and C(M)IT/MIT, CAS No 55965-84-9, which were discussed at RAC-36). RAC considers that the 48-hour time period is the most sensitive in the case of MBIT and should be considered for hazard classification as the test guideline validity criteria were met. On the basis of initial test substance concentrations, valid 48-h E_rC_{10} endpoints are within the range 0.1 to 1 mg/L. As MBIT is considered not rapidly degradable, this results in classification as Aquatic Chronic 2.

A chronic fish toxicity study is available using *Pimephales promelas* with a 32-d NOEC of 0.16 mg/L which also results in classification of Aquatic Chronic 2 for a not rapidly degradable substance. An acute ecotoxicity endpoint is not available for this species and it is unknown whether it is more or less sensitive than the most acutely sensitive fish species in the data set (*Oncorhynchus mykiss*, 96-h LC_{50} 0.24 mg/L). On this basis it is appropriate to consider the surrogate approach using the acute endpoint for *O. mykiss*. This would result in classification as Aquatic Chronic 1 for a not rapidly degradable substance, with an M-factor of 1. However, a

chronic test guideline method is not available for *O. mykiss*, so RAC does not consider it is appropriate to take a more stringent approach than suggested by all the other data.

The chronic *Daphnia* NOEC is in the same concentration range as for algae. However, mysid shrimp are the most acutely sensitive invertebrate species (96-h LC_{50} = 0.48 mg/L), but chronic toxicity data for mysids are not available. Using the surrogate approach, this could imply that the substance is classifiable as Aquatic Chronic 1. However, the acute-to-chronic ratio for *Daphnia magna* would result in an estimated chronic mysid NOEC of around 0.2 mg/L which would fall in the same classification range as the algal data. RAC therefore considers that this data gap does not impact the classification.

In summary, on the basis of the available data, RAC considers that MBIT should be **classified as Aquatic Chronic 2, H411; Toxic to aquatic life with long lasting effects**. This is consistent with the conclusion of the DS, but follows a different line of reasoning with regard to rapid degradation and the interpretation of the algal studies.

Additional references

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ANNEXES:

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

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