Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-methylisothiazol-3(2H)-one (ISO)

EC number: 220-239-6
CAS number: 2682-20-4

CLH-O-0000001412-86-105/F

Adopted

10 March 2016

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CLH-O-0000001412-86-105/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 (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-methylisothiazol-3(2H)-one (ISO)

EC number: 220-239-6

CAS number: 2682-20-4

The proposal was submitted by **Slovenia** and received by the RAC on **6 July 2015.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS). The classification notation for 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer provided.

PROCESS FOR ADOPTION OF THE OPINION

Slovenia has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **14 July 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 August 2015.**

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-rapporteur, appointed by RAC: **Pietro Paris**

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

The RAC opinion on the proposed harmonised classification and labelling of 2-methylisothiazol-3(2H)-one was reached on **10 March 2016** and was adopted by **consensus**.

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

Annex VI	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 Entry	**No current entry in Annex VI**										
Dossier submitter proposal	TBD	2-methylisothiazol-3(2H)-one (ISO)	220-239-6	2682-20-4	Acute Tox. 2 Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H311 H301 H314 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H311 H301 H314 H317 H410	EUH071	Skin. Sens 1A; H317: SCL ≥ 0.06 % M=10 M=1	
RAC opinion	TBD	2-methylisothiazol-3(2H)-one (ISO)	220-239-6	2682-20-4	Acute Tox. 2 Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H311 H301 H314 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H311 H301 H314 H317 H410	EUH071	Skin. Sens 1A; H317: SCL ≥ 0.0015 % M=10 M=1	
Resulting Entry	TBD	2-methylisothiazol-3(2H)-one (ISO)	220-239-6	2682-20-4	Acute Tox. 2 Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H311 H301 H314 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H311 H330 H314 H317 H410	EUH071	Skin. Sens 1A; H317: SCL ≥ 0.0015 % M=10 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-Methylisothiazol-3(2H)-one (MIT) is an active substance used in biocidal products as a preservative and slimicide. It is marketed under different commercial names. During the Public Consultation, 50 comments were received; 44 related to human health, one to physical hazards and the remaining 5 related to the environmental endpoints. They were provided by an EU expert scientific committee, companies that manufacture MIT, Member States, groups of expert clinical scientists and by private individuals.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and as such, MIT does not meet the criteria for classification.

Comments received during public consultation

One industry organisation agreed with the assigned physico-chemical properties, based on the available data.

Assessment and comparison with the classification criteria

The screening procedures applying structural examination, oxygen balance calculation and available thermodynamic data indicated that the active substance is not considered explosive. MIT has no functional groups capable of being oxidised and a test using EC method A.10 showed that MIT was not highly flammable. Therefore RAC is in agreement with the DS that **classification is not required for physico-chemical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS summarised seven acute toxicity studies in the CLH report, covering oral (rat, mouse), dermal (rat) and inhalation (rat) route of exposure.

In four acute oral toxicity studies, mortalities, clinical signs and necropsy findings (reddened mucous membrane of the stomach and reddened intestines) were consistently observed in rats and mice. MIT was found to be toxic by the oral route. The DS concluded that MIT should be classified as Acute Tox. 3; H301 (Toxic if swallowed) on the basis of the lowest LD_{50} of 120 mg MIT/kg bw (female rat), because this LD_{50} is within the limits 50 mg/kg < LD_{50} ≤ 300 mg/kg.

Two acute dermal toxicity studies were conducted in Wistar or Crl:CD®BR rats. The DS reported a large discrepancy in the LD_{50} values between both studies. In the study with Crl:CD®BR and pure MIT (97.5% a.s.), exposure to MIT induced mortalities, skin blanching, oedema, darkened areas, eschar formation, sloughing of skin and gastrointestinal changes observed at the necropsy. According to the DS, female rats were more sensitive than males but the study design didn't allow determination of an LD_{50} in females. An LD_{50} of 242 mg MIT/kg bw was derived for males only. The second acute dermal toxicity study conducted in Wistar rats showed no mortality after contact of MIT with skin, despite a higher applied dose of MIT/kg bw (2000 mg/kg). Taking into account the worst case scenario (i.e. the more conservative result), the DS concluded that MIT shall be classified as Acute Tox. 3; H311 (Toxic in contact with skin) on the basis of the lowest LD_{50} of 242 mg MIT/kg bw (male rat), because this LD_{50} value is within the limits 200 mg/kg < LD_{50} ≤ 1000 mg/kg.

In all three acute inhalation toxicity studies (4-hour nose-only exposure), MIT caused mortalities, laboured breathing, dyspnoea, increased respiration rate, reddish discharge around the nose and redness of lung lobes and red pin point foci on the lungs of rats. Signs of respiratory irritation were observed in these studies. Very consistent results were obtained in these studies despite the fact that they were conducted with different MIT purities or product types. The DS concluded that MIT should be classified as Acute Tox. 2; H330 (Fatal if inhaled) on the basis of the lowest LD_{50} 0.11 mg MIT/L air (rat), because this LD_{50} is within the limits 0.05 mg/L/4h < LD_{50} ≤ 0.5 mg/L/4h.

Since the mechanism of pulmonary toxicity is considered to be corrosivity, the DS also proposed labelling MIT with the additional labelling phrase EUH071 "corrosive to the respiratory tract".

Comments received during public consultation

Oral toxicity

Two manufacturers and one member state competent authority (MSCA) agreed that Acute Tox. 3 is appropriate for MIT.

Dermal toxicity

One manufacturer and one MSCA considered Acute Tox. 3 to be appropriate for MIT. A second manufacturer disagreed with the proposal for Category 3. The company considered that the large discrepancy in the LD_{50} values between the 2 studies (242 mg /kg vs 2000 mg/kg) could be explained by the different states of aggregation of the active substances that were tested in the two different studies. The first study (LD_{50} = 242 mg/kg bw) used the solid/neat substance (97.5%) wetted with vehicle, whereas the second study (LD_{50} > 200 mg/kg bw) used a technical watery solution (49% a.i.). On this basis, the manufacturer suggested a "split entry classification" to recognise the two different conditions of aggregation of MIT leading to different results in respect of dermal toxicity.

The DS had understood that split entry classifications are not possible, and did not agree that the different states of aggregation of the active substance could explain the discrepancy in LD_{50} values since both studies were performed with MIT in the same vehicle (water).

Inhalation

One MSCA commented on acute toxicity, agreeing with the proposed classification as Acute Tox. 2. In contrast, a manufacturer questioned the relevance of data obtained by means of an aerosol in view of the low vapour pressure of the substance and of the intended and reasonably expected conditions of handling and use of the substance. They did not agree that classification was warranted for this endpoint.

Given that the effects observed in the acute inhalation study were primarily due to the irritant/corrosive nature of the test material and because the potential for inhalation exposure to the technical material is considered negligible, another manufacturer questioned the need for classification for acute inhalation toxicity. The manufacturer commented that like all isothiazolones, MIT causes local, route of exposure-related effects. In obligate nasal breathers such as rats, the local effects result in asphyxiation caused by accumulation of exudates in the airways.

In response, the DS explained that in the acute inhalation toxicity studies, severe effects, including mortalities that could result from corrosive properties of MIT were observed and cannot be neglected. The results of these three well-conducted studies justify a category 2 classification for acute inhalation toxicity.

EUH 071

Based on mechanistic considerations, one manufacturer disagreed with the proposed inclusion of the EUH071 phrase.

Assessment and comparison with the classification criteria

Oral

Following exposure to MIT by gavage in 4 studies (3 in rats and 1 in mice), LD_{50} values of 120 – 328 mg MIT/ kg bw were established. All but one of these values fall within the range 50 < ATE ≤ 300mg/kg bw and therefore RAC is of the opinion that MIT meets the criteria for classification with Acute Oral Toxicity Category 3.

Dermal

In the first study, in which the rats were exposed to MIT (97.5% a.s.), the LD_{50} value was found to be 242mg/kg bw. Blanching, oedema, darkened area, eschar, sloughing, scabbed areas were observed. Effects on the skin persisted until day 14 when the study was terminated. Furthermore, gastrointestinal changes were observed at necropsy. In the second study, the rats were exposed to diluted MIT (49.0% a.s.). Irritation was observed but there were no reports of systemic effects. In this study, the LD_{50} value was > 2000mg MIT/kg/bw. The reason for large discrepancy between the LD_{50} values found in the different studies was not explained by the DS but the different concentrations of MIT may have been a contributing factor. There is no firm basis to disregard either of these values. Therefore, in accordance with the criteria, the harmonised classification should be based on the lower value.

As a result of the LD_{50} of 242mg/kg bw, RAC is of the opinion that MIT meets the criteria for classification with Acute Dermal Toxicity Category 3 (200 < ATE ≤ 1000mg/kg bw).

Inhalation

Three acute inhalation studies are available, all of which involved exposure of rats to aerosols of MIT. The following LC_{50} values were obtained: 0.11, 0.19 and 0.134 mg/L MIT. They are all within the range (0.05 < LC_{50} ≤ 0.5 mg/L) given in the criteria for classification in Acute Inhalation Toxicity Category 2 for dusts and mists. Two manufacturers commented that the exposure conditions in these laboratory studies were unrealistic compared to normal handling and use conditions. However, RAC is of the opinion that the results show an inherent potential for acute toxicity. In accordance with the criteria in the CLP Regulation, which indicate that classification should be based on the intrinsic hazardous properties of a chemical, the data cannot be over-looked or "downgraded" for classification purposes.

Clinical signs observed during the acute inhalation studies were consistent with respiratory irritation/corrosion. These included gasping, rales, laboured breathing, respiratory noise, salivation, red stained muzzle and eyes and nasal exudate. Necropsy revealed signs of slight to severe redness in the lobes of the lung in all the groups. Scattered incidences of red pinpoint foci on the lungs and gas-filled stomachs were also observed.

Given that MIT is corrosive to the skin and eyes (see the STOT SE section below), RAC considers the most likely explanation for the observed inhalation toxicity is its corrosive nature. On this basis, although the DS and those who responded during the public consultation did not consider the potential for other mechanisms of toxicity, it seems reasonable to conclude using expert judgement that EUH071 *("Corrosive to the respiratory tract")* should be applied.

In conclusion, RAC agrees with the DS that classification as **Acute Tox. 3: H301 – Toxic if swallowed, as Acute Toxicity 2: H330 – Fatal if inhaled and as Acute Toxicity 3: H311 – Toxic in contact with skin is warranted for MIT**. In addition, RAC is of the opinion that the additional labelling phrase **EUH071: Corrosive to the respiratory tract** is justified.

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

Summary of the Dossier Submitter's proposal

MIT is a corrosive substance and can therefore be considered as a respiratory irritant as indicated by the results of acute inhalation toxicity studies in rats. MIT was also evaluated in mice using the upper airway irritation test which is a measure of sensory irritation (standard method: ASTM E981-84). The results of that study showed an exposure concentration producing a 50% respiratory rate (RD_{50}) decrease of > 157 µg MIT/L.

RAC note: 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.

Overall, based on the results from acute inhalation toxicity studies in rats, supported by an upper airway irritation test in mice and considering the corrosive properties of MIT, the DS indicated that MIT could be classified as STOT SE 3, H335. However, as the effects are accounted for by the classification for acute inhalation toxicity (Acute Tox. 2) and the application of the EUH071 phrase, the DS did not propose classification and labelling for STOT SE.

Comments received during public consultation

One manufacturer supported classification of MIT with STOT SE 3, H335 (may cause respiratory irritation).

The DS responded that since EUH071 is assigned to MIT, the classification STOT SE 3, H335 is redundant.

Assessment and comparison with the classification criteria

From the acute toxicity studies following oral, inhalation or dermal exposure there was no clear evidence of (non-lethal) effects on a specific target organ or tissues. RAC considers that classifications for acute toxicity and corrosivity (see next section) cover MIT toxicological effects. An additional classification as STOT SE 1 or 2 is therefore not appropriate.

The hazard class STOT SE 3 should cover 'transient' respiratory tract irritation and narcotic effects that are observed in animal studies. Lethargy, lack of coordination, loss of righting reflex and ataxia occurring after single exposure can justify classification of substances for narcotic effects in Category 3. Classification in Category 3 is primarily based on human data which is not available for MIT.

Although the data suggest that MIT is a respiratory irritant, the effects are accounted for by the classification for acute inhalation toxicity (Acute Tox. 2) and the application of the EUH071 phrase. Therefore, RAC agrees with the DS that additional **classification and labelling for STOT SE is not warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS considered MIT to be corrosive to skin and eyes (the eye irritation potential of MIT was not tested since the substance is corrosive to the skin) based on the corrosive effects observed in rabbits exposed to MIT for 3 minutes (6 animals average erythema score 1.0, oedema score: 0.4, erythema persisted for 7 days), 1 hour and 4 hours (erythema and oedema score 4.0, erythema irreversible after 7 days) and corrosiveness in an *in vitro* human skin epidermal construct study (EPIDERM, EPI-200) in accordance with OECD TG 431. The EPIDERM study demonstrated corrosivity since after 60 minutes exposure to 51.5% MIT, a reduction of cell viability to 13.6% was observed.

Based on the dose selection used in submitted skin irritation/corrosion studies, the SCL for MIT cannot be derived. Therefore the generic concentration limit (< 1% w/w) will apply for the mixtures.

The DS concluded that MIT should be classified as Skin Corr. 1B, H314 (Causes severe skin burns and eye damage) based on the corrosive effects observed in rabbits exposed to MIT for 3 minutes, 1 hour and 4 hours and corrosiveness in a human skin epidermal construct.

Comments received during public consultation

One manufacturer agreed with the proposed classification as Skin Corr. Category 1B; H314.

One MSCA considered that the data presented in the CLH report are not sufficient to support classification of MIT as Skin Corr. 1B. Although erythema was still noted after a 14 day observation period following 1 hour exposure, no clear corrosive responses indicating destruction of skin tissue (e.g. visible necrosis) were described in the *in vivo* studies. On this basis, the MSCA proposed classification of MIT as Skin Irrit. 2; H315. A second MSCA requested further information on the effects indicating corrosivity because the irreversibility of erythema is not determinative for classification as corrosive, but only for category 2. In response, the DS explained that the skin irritation scores in the rabbit studies justified a category 1B classification (see '*Additional Key Elements*' section).

The second MSCA also considered that an occupational accident described in the skin sensitisation section is more relevant to the discussion on irritation/corrosion. The DS agreed with the suggestion. The incident was described as follows: "*An accident with MIT was reported when one of the workers in Rohm and Haas was exposed to the substance. In this case blistering and*

reddening of skin were the signs of exposure. Over the years of manufacturing MIT, no worker has experienced continuing skin problems and none has had to be transferred to other duties due to exposure to chemicals."

Assessment and comparison with the classification criteria

Two skin irritation studies (both in rabbits) are available.

In the first study, erythema was observed with average scores of 1, 4 and 4 after exposures of 3 mins, 1 hour and 4 hours, respectively. This effect was irreversible. As summarised above ("Additional Key Elements"), blanching and eschar, which are considered to be indicative of corrosivity, were observed in conjunction with erythema after 1 hour exposure to MIT. Oedema, which was reversible, was also observed, with average scores of 0.4, 4 and 4 after exposures of 3 mins, 1 h and 4 h, respectively.

RAC notes the somewhat limited reporting of this study. Scores for individual animals were not available and there were no data for observations 14 days after patch removal following 1 h exposure to MIT. However, high scores were reported from just 1 h after patch removal (erythema: 3; oedema: 4). Moreover, the irritation scores and skin findings were almost identical following 1 and 4 hours exposure. Therefore it is reasonable to expect erythema and related skin findings to persist until day 14 following 1 h exposure, as it did following 4 hours exposure. The study is considered to indicate that MIT is corrosive.

In the second study, following 4 h exposure to MIT, erythema and oedema were observed with average scores of 2 and 0.77, respectively, 24, 48 and 72 h after exposure. Due to eschar formation, oedema was not evaluated on days 7, 10 and 14. Erythema, with a score of 4, was observed from days 7-14. The observation of eschar as the study progressed is indicative of dead tissue formation following corrosive damage.

The results of these 2 studies indicate that MIT exposure for 1h or 4h can produce a corrosive effect on rabbit skin. They do not provide information on the potential corrosivity of MIT following shorter exposure periods.

The results of the *in vitro* EPIDERM study support subcategorisation of corrosive substances into Category 1A, but discrimination between Categories 1B and 1C is not possible. According to the relevant test guideline, "corrosive chemicals are identified by their ability to decrease cell viability below threshold levels." When ≥ 50% of cells are viable after 3 minutes exposure and < 15% of cells are viable after 60 minutes exposure, this is considered to indicative of corrosivity and support classification with a combination of subcategories 1B and 1C. In the study described in the CLH report, MIT was used at concentrations of 1.7% and 51.5% in water. No corrosive response was evident at 1.7%. At 51.5%, MIT was not corrosive after 3 minutes exposure. However cell viability was reduced to 13.6% following 60 minutes exposure. This result provides supportive evidence for classification of MIT as Skin Corr. 1B or 1C.

RAC notes that the case report of a workplace accident with MIT provides limited information about the corrosive potential of MIT. No firm conclusion on corrosivity can be derived from this information. However, the case report is not inconsistent with MIT being corrosive.

After 1h and 4h dermal exposure, observations of severe erythema (progressing to score 4 and not reversible) and the blanching of skin with eschar formation are collectively considered to be evidence of corrosivity. RAC agrees with the DS that MIT meets the criteria for classification as

Skin Corr. 1B; H314 (Corrosive in >1 of 3 animals following exposure > 3 minutes - ≤ 1 hour, with an observation period of ≤ 14 days).

In summary, based on the weight of evidence, RAC agrees with the DS proposal to classify MIT as **Skin Corr. 1B**. The results of the *in vitro* human epidermal construct study are considered to support this classification.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Eye irritation potential of MIT was not tested since MIT is corrosive to the skin and therefore it is considered to be corrosive also to the eye. In accordance with the Technical Notes for Guidance on data requirements, MIT was not tested for eye irritation.

Comments received during public consultation

No comments were received addressing this endpoint.

Assessment and comparison with the classification criteria

According to the CLP criteria, skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). Since the data are considered sufficient to classify MIT as Skin Corr. 1B, it is reasonable to assume that MIT would also damage the eyes. However, **classification for eye corrosion/irritation is not proposed** since this hazard is covered by the hazard statement for Skin Corr. 1B (H314: Causes severe skin burns and eye damage).

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

Respiratory sensitisation of MIT was not tested.

Several cases of airborne allergic contact dermatitis and systemic contact dermatitis have been observed and assumed to result from the airborne exposure to MIT from recently painted walls. Reported cases of airborne contact dermatitis were not confirmed by patch testing with the paints.

The information does not allow a conclusion on the respiratory sensitisation potential of MIT to be drawn.

Comments received during public consultation

A manufacturer considered that MIT does not fulfil the criteria for respiratory sensitisation.

Assessment and comparison with the classification criteria

The data presented by the DS are relevant to the endpoint skin sensitisation, not respiratory sensitisation. RAC agrees that there is **no basis for classification of MIT as a respiratory sensitiser**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Dossier Submitter proposal submitted in the CLH report

MIT has been shown to be a skin sensitiser in a local lymph node assay, Buehler test, the Guinea Pig Maximisation Test (GPMT) of Magnusson and Kligman and in an open epicutaneous test.

In the local lymph node assay, the stimulation index was above 3 (6.65) at an MIT concentration of 1.35%, which fulfils the criteria for Skin Sens. 1A (EC3 value ≤ 2%).

After induction with 0.1% MIT in a GPMT, signs of skin sensitisation were observed in 10/10 animals. This fulfils the criteria for Skin Sens. 1A, where ≥ 30% should respond positively after induction with concentration ≤ 0.1%.

Therefore, the DS proposed that MIT should be classified Skin Sens. 1A, H317 (May cause an allergic skin reaction)**.**

The human repeated insult patch test (HRIPT) has been used to test the skin sensitisation potential of solutions containing different MIT concentrations. A sensitisation response was found in 1/116 and 1/210 volunteers exposed to 400 ppm (0.04% or 20 µg/cm^2) or 500 ppm (0.05% or 25 µg/cm^2), respectively. At lower concentrations (0.01% or 3.75 µg/cm^2, 0.02% or 10 µg/cm^2, 0.03% or 15 µg/cm^2) and at the higher concentration of 0.06% (or 30 µg/cm^2) MIT did not induce skin sensitisation after 9 consecutive applications followed by 10-15 days rest before challenge. The study was designed to maximise exposure to the test substance, the exposure being repeated nine times over a 21 day period with occlusion. In addition, the study used a formulated product (50% in propylene glycol) diluted in water. This may affect the sensitisation potential due to vehicle effects. Given the lack of a clear dose-response in this study, the DS concluded that its suitability for defining a specific concentration limit (SCL) is questionable.

Skin sensitisation after exposure of contact dermatitis patients to MIT has been reported in clinics from several European countries. Some case reports on allergic reactions to MIT have also been published. The scientific robustness of these data and their suitability for classification purposes is questioned, as many of the reports were not peer reviewed, adequate reporting and presentation of data is lacking, and exposure was not sufficiently characterized.

Based on skin sensitisation studies in animals and the skin sensitisation study in humans setting SCL for skin sensitisation 0.06% seems justified, which is lower than the generic concentration limit (GCL) for a skin sensitiser in category 1A.

The proposed SCL may not be protective enough for some MIT pre-sensitised individuals as indicated in published clinical studies. Accordingly, EUH 208 – Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction should be applied for mixtures not being classified for skin sensitisation, but containing more than 0.006% of MIT.

Revised position of the Dossier Submitter following the Public Consultation

After the public consultation, the DS confirmed that MIT should be classified Skin Sens 1A, H317, but that the data and weight of evidence analyses submitted during the public consultation show that the proposed SCL of 0.06% (600 ppm) may not be sufficiently protective for sensitised

individuals. Therefore, the DS responded that a lower limit should be defined. The DS emphasised that all mixtures containing MIT should be automatically labelled with EUH208: "Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction."

Comments received during public consultation

Comments were received from 3 manufacturers of MIT, 7 MSCA, 6 scientific bodies, an additional non-governmental organisation, two private individuals and the EU Scientific Committee on Consumer Safety (SCCS).

1. Hazard assessment and classification Skin Sens. Cat 1A, H317

All those who commented on this aspect of the proposal were supportive of the classification Skin Sens. Cat 1A, H317.

One manufacturer described how the results of the LLNA assays (EC3 values of 04% and above 0.76%), the maximisation tests (26% positive response at 0.1% MIT and 100% positive response at 1% MIT, respectively) and the Buehler assay (60% positive response at 1.5%) all justified classification in Category 1A. They also commented on how the total number of cases of MIT sensitisation reported across the EU was sufficient to conclude MIT was a potent sensitiser.

One MSCA elaborated how the criteria for CLP stipulate that all relevant information should be taken into account in assessing the skin sensitisation potential of MIT, including information published in peer reviewed scientific journals, from medical authorities and dermatological clinics.

2. Derivation of a SCL for Skin Sens. Cat 1A, H317

A second manufacturer commented that the use of MIT in their product types has resulted in an extremely rare amount of skin reactions and therefore they consider that the GCL of 0.1% is adequate. A second manufacturer considered that the GCL of 0.1% should be applied, as this is standard for high potency sensitisers. In support of this, they noted how the available animal data indicated that MIT was a strong sensitiser, not an extreme sensitiser. They cited the CLP guidance that stated SCLs can only be set on the basis of testing of the substance and never a mixture. In their opinion, the prevalence and clinical data indicated high potency, but could not be considered sufficiently reliable for setting a SCL. They acknowledged that the use of HRIPT data may be used in a weight of evidence approach to support subcategorisation, but argued that the methodology of the HRIPT test (repeated treatments for 21 days with occlusion and the use of water as a vehicle to dilute a test sample that was a formulation of MIT in polyethylene glycol) produced an extreme exposure scenario and should not be used in isolation to justify a lower SCL.

A third manufacturer commented that the available animal studies, including the LLNA, GPMT and Buehler tests, showed MIT to be a potent sensitiser. In their opinion the human prevalence and clinical data also pointed to MIT being a potent sensitiser, but these data were not sufficiently reliable for setting an SCL since the exposures that had caused induction in the affected individuals had not been defined. Similarly, repeated open application test (ROAT) data from already sensitised patients were not suitable, as they related only to challenge, not induction concentrations. The only relevant human data came from the HRIPT, where exposure was carefully controlled. However, the study with MIT was not considered sufficiently robust for regulatory purposes, and the third manufacturer preferred that the GCL of 0.1% for a potent sensitiser be applied to MIT.

In contrast, all seven MSCA that commented did not support the setting of a SCL of 0.06% (600 ppm). Generally, they did not follow the argumentation provided by the DS. Concern was expressed about the recent rapid increase in incidence of MIT induced allergies observed in several European countries. The data from a large number of dermatological clinics over the past 5-10 years clearly indicate an increased prevalence of allergic contact allergy to MIT. This appears to be related to the widespread use of cosmetics that have been present on the market containing 100 ppm MIT or less during this period. Data were also available suggesting that other products that contain this level of MIT, including water-based decorative paints and various other household products, may induce skin sensitisation in consumers. The Danish Coatings and Adhesives Association, for example, had reported that 80% of water-based paints from their members contained less than 100 ppm MIT; 19% contained between 100 and 200 ppm. Further, a survey across the EU had shown that MIT was present in all but 5 of the 71 assessed paints: 18% of these contained < 15 ppm MIT, 45% between 15 and 100 ppm and 30% contained over 100 ppm. All this information led these MS to conclude that an SCL of 600 ppm would be too high for MIT.

Four MSCA cited the findings of Yazar et al. (2015), a study published after the CLH report had been submitted. They commented that this study had employed the repeated open application test (ROAT) to show how rinse-off cosmetic products containing 100 ppm or 50 ppm MIT are not safe. It was proposed that the SCL should therefore be below 50 ppm.

One MSCA proposed a SCL of 10 ppm (0.001%). This was based on a "No expected Sensitisation Induction Level" (NESIL) of 10 ppm in HRIPTs on MIT and the related reaction mass of 5-chloro-2-methyl-4-isothiazolin-3-one and MIT (3:1) or C(M)IT/MIT (3:1), as derived by the SCCS in 2014. In support of this, a recent report from the UK (Warburton and Wilkinson, 2015) had indicated that new cases of MIT sensitisation might still be possible if the concentration of MIT in rinse-off cosmetics is limited to 15 ppm (RAC note: no further explanation was provided in the comment).

Three MSCA instead proposed an SCL of 15 ppm (0.0015%). According to two of these MSCA, the SCCS had considered this level safe for MIT in rinse-off cosmetic products. One MSCA indicated specifically that the analysis of available animal and human data from this group of EU scientific experts should be taken into account in the setting of an SCL for MIT, in order to avoid a duplication of work. Another MSCA commented that this was the SCL already in place for the related substance C(M)IT/MIT, and it therefore seemed appropriate to also set this SCL for MIT.

Regarding the SCCS opinion (SCCS, 2015), the second manufacturer of MIT described above observed that this focussed on the prevention of elicitation in already sensitised individuals rather than the prevention of induction. Many of the human studies were not suitable for limit setting as they were not peer reviewed, reporting was inadequate since presentation of data was lacking, and the exposure levels causing induction were not sufficiently characterised.

Responding to these comments, the DS acknowledged that the proposed SCL of 600 ppm was not well justified. They also concluded that 0.1% was not sufficiently protective, acknowledging the studies reporting an increasing incidence of confirmed MIT sensitised individuals, skin sensitising reactions to MIT at concentrations below 600 ppm and wide use of MIT in industrial and consumer products.

In their comments; the SCCS agreed that an SCL is needed, citing both the results of the HRIPT with MIT, the unusually high number of sensitisation cases to MIT, and reported increase in numbers up to 6-fold among consumers and workers patch tested for MIT sensitisation in different

areas of Europe. Although it had ethical concerns about the HRIPT in general, the results of the HRIPT with MIT appeared to show induction responses to levels as low as 100 ppm. Although a clear dose-response relationship was not observed, there were no scientific reasons to specifically disregard the results from the lower dose groups. The results could not be explained by the irritant nature of MIT and were more likely a consequence of the challenge dose not being sufficiently high.

The SCCS also stated that:
- It is well known that cosmetic products with up to 100 ppm carry a significant risk of sensitisation.
- Paints are frequent causes of MIT sensitisation in workers and also in consumers. Currently the majority of water-based paints contain MIT in concentrations below 100 ppm.
- In other chemical products for consumer and occupational use, concentrations below 100 ppm of MIT are in use.
- Having assessed the risk of MIT in rinse-off cosmetic products, the committee had concluded previously that 15 ppm would be safe for the consumer from the view of induction of skin sensitisation.

Further, SCCS noted that the related C(M)IT:MIT (3:1) is also a potent skin sensitiser used as a biocide. The SCCS observed that it already has a harmonised SCL of 15 ppm in Annex VI of the CLP Regulation (Skin Sens. 1; H317: C $\geqslant$ 0,0015 %) and proposed that this limit was also justified for MIT. The frequency of allergic reactions to C(M)IT:MIT was stable over many years around 2% of patch tested contact dermatitis patients. But after the introduction of MIT as a stand-alone biocide a rapid increase in contact allergy was seen not only to MIT, but also to the reaction mass C(M)IT/MIT. This is obviously likely to be explained by MIT being present in C(M)IT:MIT and to chemical similarities between the substances (C(M)IT and MIT), so that exposure to one may result in cross-reactivity to the other. Thus, according to the SCCS, the substances should therefore be treated identically with respect to setting the SCLs.

The European Society of Contact Dermatitis, the Swedish Contact Dermatitis Research Group, the Swedish Institute of Environmental Medicine, the Finnish Institute of Occupational Health and a statement issued on behalf of over 140 experts from dermatology, allergenicity, epidemiology, occupational medicine and health education across the EU, also found strong indications that MIT concentrations below 600 ppm will sensitise people. They suggested an SCL of 15 ppm.

3. Labelling of mixtures with phrase EUH208:
"Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction."

The third manufacturer commented that a limit of 0.01% should be applied for EUH208, this being 10-fold lower than the general classification limit of 0.1% that they felt was justified. The other two manufacturers did not comment on EUH208.

However, other comments sought a much lower limit for labelling with EUH208. In some cases, including from the SCCS and one member state, no limit at all was recommended. It was suggested that products containing MIT should be labelled with the name of the sensitising substance, but with no lower concentration limit because they considered that the criteria in CLP Annex II to label sensitisers with EUH208 down to 1/10 of the GCL or SCL is not sufficiently protective to prevent elicitation of allergic contact dermatitis in those already sensitised to MIT.

The Swedish Institute of Environmental Medicine and a statement issued on behalf of over 140 experts from dermatology, allergenicity, epidemiology, occupational medicine and health

education across the EU, also commented that no limit should be set for the application of EUH208 to MIT.

The European Society of Contact Dermatitis, like some of the commenting MSCA, observed how in a recent repeat open application test (ROAT) with 2 liquid hand soaps preserved with MIT at 100 ppm and 50 ppm, all volunteers sensitised to MIT tested positively to 100 ppm and 78% reacted to 50 ppm (Yazar *et al*., 2015). Thus, labelling at 0.006% (60 ppm) will not protect individuals already allergic to MIT. This body recommended a limit of 1 ppm for the additional warning label. The Swedish Contact Dermatitis Research Group made a similar comment, but recommended no lower limit. They cited a recent Finnish study claiming that MIT exposure levels in products that have sensitised and elicited allergic contact dermatitis in patients are in the range 10-21 ppm (Vauhkala *et al*., 2015). The Finnish Institute of Occupational Health commented that in this study a total of 33% of the patients had used MIT or c(M)IT/MIT-containing products without any mention of these substances in safety data sheets or product declarations. This body also saw the need for a low labelling limit.

Assessment and comparison with the classification criteria

RAC agrees with the DS and all those parties that commented during the public consultation that MIT is a potent sensitiser. This is shown both by the results of animal studies and the available human data.

As shown in the following table, GPMT, Buehler and Local lymph node assays have been conducted with MIT, all providing results that match the criteria for classification in sub-category 1A. In the additional LLNA , the EC3 value of between 1.25 and 2.5% MIT provides further support for this classification.

Animal test	**Criteria for high potency (sub-category 1A)**	**MIT data**	**Conclusion**
Guinea pig maximisation test (Thor study)	≥30% responding at ≤0.1% or ≥60% responding at >0.1% to ≤1% intradermal induction concentration	100% response at 0.1% intradermal induction concentration of MIT (observed at 1% challenge concentration)	The response rate at an intradermal induction concentration of 0.1% meets the criteria for Cat. 1A.
Guinea pig maximisation test (Rohm and Haas study)	≥30% responding at ≤0.1% or ≥60% responding at >0.1% to ≤1% intradermal induction concentration	26% (5/19) response rate at 0.08% and 20% response rate at 0.055% intradermal induction concentrations (observed at 2nd challenge; 0.1% MIT)	The response rates were not sufficiently high to meet the criteria for Cat. 1A. However, induction concentrations of 0.08 and 0.055% MIT were below the 0.1% value and it is unclear if a response rate of 30% would have been seen at that concentration. The data therefore do not necessarily indicate a lack of high potency.
Buehler test (Rohm & Haas study)	≥15% responding at ≤0.2% or ≥60% responding at >0.2% to ≤20% topical induction concentration	≤10% response at 0.1% ≤60% response at 0.5% ≤30% response at 1.5% ≤50% response at 3% topical induction concentrations of MIT	The response rate at a topical induction concentration of 0.5% meets the criteria for Cat. 1A

		(increasing response rates were seen as the challenge concentration was increased from 0.1, 0.5 and 1.5% MIT).	
Local lymph node assay (Rohm & Haas study)	EC3 value ≤2%	EC3 value at 0.86%	The EC value meets the criteria for cat. 1A

In humans, the repeat insult patch test (HRIPT) has been conducted with MIT. RAC notes the ethical concerns about the use of these tests expressed during the public consultation; the tests date back to 2000-2001 and were not performed specifically to address the classification of MIT under the CLP Regulation. The test subjects were given repeated dermal exposures for 9 consecutive days, followed by 10-15 days of rest. Challenge was then performed at the same concentration of MIT used for induction. The sensitisation rates observed were 1/98 (1 volunteer was pre-sensitised), 0/100, 0/98, 1/116, 1/210 and 0/214 at 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% MIT, respectively. These concentrations tested resulted in exposures ranging from 3.75 to 30 μg/cm^2, levels well below the value of 300 μg/cm^2 described in the CLP Guidance on the Application of CLP Criteria (Version 4.1 – June 2015) as a cut-off relevant for the sub-category 1A.

The DS queried the relevance of the remaining human data for classification purposes, mainly because the studies were not conducted as rigorously as standard regulatory tests in animals. It was questioned whether the information from dermatology clinics and in case reports was sufficiently robust to be used in the classification process. However, the DS later responded to the public consultation, by commenting that thisshould be taken into account in the weight of evidence.

As indicated by the SCCS, other scientific bodies and several MS during the public consultation (see above), the consistency of the human clinical data and information about the most likely sources of exposure that could have led to those affected becoming sensitised provide a compelling case for MIT being regarded at least as a high potency sensitiser. The human data support the results of the animal tests. RAC also notes that the chemically-related substance C(M)IT/MIT (3:1), having being extensively reviewed previously, is already classified as Skin Sens. 1A in Annex VI of the CLP Regulation. Therefore, although the animal data alone would be sufficient to justify sub-categorisation of MIT in Cat. 1A, RAC's view is that the human data provide valuable supporting evidence. Specifically, there is a relatively high and substantial incidence of reactions among consumers that use cosmetics and household products (e.g. water-based paints) that contain MIT and the scale of this has been increasing in recent years.

In conclusion, RAC agrees with the proposal that classification with **Skin Sens. 1A** is justified for MIT.

Specific concentration limit and additional labelling with phrase EUH208

In response to several weight of evidence analyses submitted in the public consultation, the DS indicated that the SCL of 0.06% MIT that had originally been proposed may not be sufficiently protective for sensitised individuals. The DS proposed that a lower SCL should be set but did not elaborate further.

The Guidance on the Application of CLP Criteria (Version 4.1 – June 2015) describes how SCLs for skin sensitisation can be set based on animal test data. The tests should be on the substance itself,

not on mixtures. Substances classified as Skin Sens. 1A may be further defined as being of extreme or strong potency. The recommended SCLs for these two different classes of potent sensitiser are 0.001% and 0.1%, respectively. However, the guidance also indicates that another value could be applied if supported by reliable data. Such data could be human data for which the exposures leading to sensitisation are defined.

RAC recognises that the setting of an SCL for a substance classified as a skin sensitiser is intended primarily to account for extremely high potency and should be based on information about the exposure conditions necessary to cause (i.e. induce) sensitisation. RAC is of he opinion that a SCL should be set with the intention of protecting non-sensitised individuals rather than to protect already sensitised individuals as suggested by the DS.

To further protect individuals who are already sensitised, the additional label EUH208 is available. That is defined in Annex II section 2.8 of the CLP Regulation. It provides a warning that a mixture contains a skin sensitiser at a level below the limit for classification. The "limit for elicitation" that would apply for the provision of this additional label would conventionally be set at 0.01% for a mixture containing a substance classified Skin Sens. 1A with a concentration limit of 0.1%. For other concentration limits, the "elicitation limit" would similarly be set 10-fold lower.

In order to set a SCL for MIT, information is needed to show that it can be regarded as an extremely potent sensitiser with the potential to produce the sensitised state at a level below 0.1%.

The relevant animal data are summarised below.

Test	**Indicative criteria for extreme potency in the Guidance on Application of the CLP Regulation**	**MIT data**
Guinea pig maximisation (Thor study)	Intradermal induction concentration ≤0.1% Sensitisation rate ≥60%	100% response at 0.1% intradermal induction concentration of MIT (1% challenge concentration) **Indicates extreme potency**
Guinea pig maximisation (Rohm and Haas study)	Intradermal induction concentration ≤0.1% Sensitisation rate ≥60%	26% (5/19) response rate at 0.08% and 20% response rate at 0.055% intradermal induction concentrations of MIT (2^{nd} challenge; 0.1%) **Not indicative of extreme potency, although the sensitisation rate at the 0.1% induction level was not investigated**
Buehler (Rohm & Haas study)	Induction concentration ≤0.2% Sensitisation rate ≥60%	≤10% response at 0.1% ≤60% response at 0.5% topical induction concentrations of MIT (increasing response rates were seen as challenge concentration was increased from 0.1, 0.5 and 1.5% MIT). **Not indicative of extreme potency**
Local lymph node (Rohm & Haas study)	EC3 value ≤0.2%	EC3 value at 0.86% **Not indicative of extreme potency**

Local lymph node (Thor)	EC3 value ≤ 0.2%	1.25% < EC3 < 2.5% **Not indicative of extreme potency**

All of the studies in the table above were conducted to an appropriate regulatory standard, so a single study cannot be considered of better quality than any of the others. One GPMT showed MIT to have extreme potency whereas another GPMT was only indicative of this. In contrast, the available Buehler and LLNA tests showed MIT to have at most strong, rather than extreme, potency. Overall, the available animal data do not provide a clear picture of the potency of MIT. On this basis, as defined in the CLP Guidance (Version 4.1, June 2015), a concentration limit of either 0.1% (1000 ppm) or 0.001% (10 ppm) would seem justified. However, RAC agrees with the MS and expert groups who commented during the public consultation that the available human data should also be taken into account.

The HRIPT study described above has been criticised for the use of water to dilute the test sample (50% MIT in propylene glycol); it may have led to a false level of uptake. The numbers of individuals included in the study were small compared to the total population at risk from exposure to products containing MIT, and the number of subjects that responded to MIT was very small, but the results appear to show that MIT concentrations below 0.1% (1000 ppm) do have sensitising potential. Although its limitations were recognised, this study was used by the DS to support their original proposal for an SCL of 0.06% (600 ppm) MIT. However, RAC considers that the individuals who became sensitised at 0.04% and 0.05% MIT should not be disregarded and that an SCL would be appropriate. In this study, the exposure levels were controlled sufficiently for the data to be used for classification purposes and the findings of sensitisation at induction concentrations below 0.1% (1000 ppm) MIT support the setting of an SCL.

to Interpretation of the remaining epidemiological data is less straightforward. It is a concern that there are an unusually high number of MIT sensitisation cases and that the frequency of such cases in recent years has increased by up to 6-fold among consumers and workers that have been tested. RAC agrees with the comments made during the public consultation that this appears to be linked to the introduction of MIT as a biocide, and especially its use in cosmetics. Insufficient data are available to RAC for independent scrutiny, but it appears that MIT is generally present in these products at levels below 100 ppm, but it is not possible to relate the many cases seen to specific exposures. RAC therefore concludes that levels of MIT below 100 ppm have the potential to induce skin sensitisation.

The SCCS has recommended that 15 ppm MIT would be a safe level in rinse-off cosmetics for protection of consumers from induction of skin sensitisation. RAC has not been provided with all the data underpinning this recommendation, but notes that comments received during the public consultation show this view of the SCCS to be supported by various groups of expert dermatologists. Additionally, RAC has noted that the SCCS (2015) concluded that there is "no adequate information to suggest a safe dose of [MIT] in leave-on cosmetic products from the view of induction of sensitisation, although circa 3.8 ppm, as present in C(M)CI/MIT, may be indicative".

In RAC's opinion, sufficient information is available to conclude that MIT has extreme potency. The results of the 2 guinea pig maximisation tests are consistent with the definition given in the CLP guidance. RAC agrees with the manufacturers of MIT who commented that the available human data are not sufficiently reliable to enable the exposure concentrations at which induction can occur to be defined accurately. However, the findings from the HRIPT study in combination with the recent epidemiological information show that it is likely that levels below 100 ppm MIT will have the potential to induce sensitisation in humans.

This profile is not inconsistent with that found for the related substance C(M)IT:MIT. This complex substance includes MIT as a constituent and is also classified as Skin Sens. 1A. Based on a detailed review of the available human evidence, the Commission Working Group on the Classification and Labelling of Dangerous Substances recommended a SCL of 15 ppm. This classification is listed in Annex VI of the CLP Regulation.

Although the CLP guidance suggests a SCL of 0.001% could be set for a sensitiser with extreme potency, in RAC's opinion it would be appropriate to set the same SCL for MIT as for C(M)IT:MIT (3:1), that is 15 ppm. This was the view of several MSCA and expert groups that responded during the public consultation. An SCL of 15ppm was supported by the SCCS, although its opinion focussed mainly on elicitation rather than induction.

The data from repeat open application tests (ROAT) in humans inform on the levels of MIT that can elicit an allergic response in sensitised individuals (see "Additional key elements", above). The study by Yazar *et al*. (2015) was much cited during the public consultation. The authors of this well-conducted Swedish study showed that the elicitation threshold is below 50 ppm. The study by Lundov *et al*. (2011) also showed that 50 ppm MIT can elicit a reaction in sensitised individuals tested. Neither of these studies contradicts the recommendation to set an SCL of 15 ppm; in both cases, the studies did not confirm the lowest level of MIT that could elicit responses.

The DS also described 4 cases of allergic contact dermatitis to MIT evaporating from wall paints. It is not clear from the available information how the 4 individuals concerned first developed their sensitivity to MIT, but the observations of facial dermatitis were consistent with elicitation by airborne exposure. Such observations hint at the possible extreme potency of MIT, at least in eliciting an allergic response.

Overall, RAC is of the opinion that the limit for application of the labelling phrase EUH208 should be as defined in Annex II of the CLP regulation, i.e. 10-fold below the SCL for classification. The limit for EUH208 would therefore be 1.5 ppm. RAC notes that some comments made during the public consultation proposed that the increasing numbers of allergy patients being found sensitive to MIT was sufficiently justified to create a special additional labelling phrase with no limit. However, RAC did not find this argument persuasive; no indication was provided to show why 1.5 ppm as derived by applying EUH208 would not be sufficiently protective for sensitised individuals.

RAC is of the opinion that a SCL is justified for MIT and should be set at 0.0015%. In accordance with Annex II of CLP, a 10-fold lower limit should apply for the additional labelling phrase EUH208.

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

Summary of the Dossier Submitter's proposal

The DS summarised four studies involving repeated oral exposure of rats and dogs up to 90 days of exposure.

In a 28-day oral toxicity study in rats (gavage) deaths occurred and lethargy, reduction in the weekly body weight gain and feed consumption during the experiment was observed. However, gross and histopathological findings observed were not considered treatment related and were

recorded either both in control and treatment groups at comparable levels or only in a few animals without any consistent pattern and were in conformity with historical control data. The DS concluded the above effects were spontaneous/incidental findings.

Similar findings were reported in a subchronic (90-day) oral toxicity study (drinking water) conducted in rats. Effects on body weight, food and water consumption were noted at 66 and 94 mg a.i./kg bw/day in males and females, respectively (1000 ppm). There was no evidence of systemic toxicity or gross and microscopic pathology at doses up to and including 19-25 mg/kg bw/day (250 ppm). In a second repeated oral toxicity study in rats (gavage), no adverse effects were observed.

Repeated dose oral toxicity of MIT was also assessed in dogs. In both sexes decreased body weight and food consumption were observed at 40.6 to 40.9 mg/kg/day (1500 ppm).

The repeated dose toxicity of MIT by the dermal and inhalation routes was not tested.

In conclusion, the DS did not propose classification for STOT RE.

Comments received during public consultation

One MSCA commented that severe effects, including mortality, were reported in a 28 day study and a teratogenicity study at relevant concentrations for STOT RE 2. However, since the information provided on the cause of death was very limited, the MS asked for further explanation to clarify why the effects were not considered sufficient for classification.

In response, the DS provided the following information to explain why classification of MIT as STOT RE 2 was not considered to be justified.

In a 28-day oral rat study, the animals of both sexes treated with the high dose of MIT, 71 mg/kg bw/day, were lethargic during week 3 and 4. At this dose 4 animals died, 1 male and 3 females, and decreased body weight and food consumption were observed in males, while no reduction of these parameters was observed in females. Another oral repeated dose study was performed in rats. Animals were exposed to a comparable dose of MIT, 66 mg/kg bw/day, for a longer period (90 days), but no mortalities were reported, only slight reduction of body weight, food and water consumption.

One of the criteria for a classification as STOT RE 2 is the observation of a consistent and identifiable toxic effect in humans or experimental animals. Since mortalities observed in the 28 day study were not seen in the 90 day study conducted with similar dose of MIT, it cannot be concluded that the effect was consistent. Longer dosing periods would be expected to result in more severe effects. According to the criteria for STOT RE 2, clinical observations or small changes in bodyweight gain, food consumption or water intake that have toxicological importance but that do not, by themselves, indicate 'significant' toxicity do not fulfil the criteria for classification STOT RE 2. Since only slightly reduced body weight, food and water consumption were observed in 90-days rat study, classification of MIT as STOT RE 2 is not warranted. A second MSCA requested that the DS check the consistency of the data presented in the CLH report.

Assessment and comparison with the classification criteria

Four oral repeated dose toxicity studies of MIT were available (3 in rats; 1 in dogs).

In a 28 day rat study (5/sex/group, doses: 0, 10, 28.6 and 71.2 mg MIT/kg bw/day by gavage), 4 animals in the top dose group died – one male in week 2 and three females in weeks 1, 2 and 4.

Changes in organ weights in this study are considered to be incidental. Lethargy and slight reductions in bodyweight gain and food consumption were observed at the top dose. Some changes in clinical chemistry parameters were noted, however all values in the high dose recovery group were comparable to those in the control recovery group by the end of the recovery period.

The dose at which the deaths occurred (71.2 mg MIT/kg bw/day) was sufficiently low to justify classification with STOT RE. This dose was lower than the oral LD_{50} value in rats (120 mg/kg), which formed the basis of the proposal to classify MIT in category 3 for acute oral toxicity. However, there were no specific indications of systemic, repeated dose toxicity in this study and therefore no additional classification beyond that for acute toxicity seems to be justified. Also, the deaths occurring in this 28-day study occurred at doses in the LD_{50} range specified for the acute toxicity classification.

In a 90 day study in rats (10/sex/group, doses: 0, 75, 250, 1000 ppm MIT in drinking water), decreased bodyweight, food consumption and water consumption were observed and are considered to be a result of the palatability of the water. No other adverse effects were reported. There were no corresponding changes in the gross pathology or histopathology indicative of treatment-related irritation in the oral cavity, oesophagus, or gastrointestinal tract.

In a second 90 day study in rats (10/sex/group, doses: 7.52, 15 and 30 mg MIT/kg bw/day by gavage), one male in the top dose group was found dead on the 54^{th} day of the experiment. The DS considered this death to be incidental. Changes in sperm parameters were noted and are described in the reproductive toxicity section of this opinion. Increases in food consumption did not reach statistical significance and despite changes to clinical chemistry parameters, the values remained within the historical control data range. The absolute weight of the spleen increased in the low and high dose groups in males by 36% and 53.2% respectively compared to controls. The relative weight of the spleen also increased in high dose males, although no significant observations were made upon histopathological examination of the spleen. In the absence of histopathological evidence to support an adverse effect on the spleen, the change in spleen weight is not considered sufficient to warrant classification. Hypocellularity, hypercellularity, lymphoid hyperplasia and eosinophilic hyperplasia were observed by smear examination of the bone marrow. The DS did not consider these effects to be treatment-related. However, there is no information available in the CLH report to allow an independent assessment of these findings.

In a 90 day study in dogs (4/sex/group, doses 0, 100/130, 400, 1500ppm in the diet, 50% MIT in water), decreases in food consumption (first 2 weeks of study only) and bodyweight were observed at 1500 ppm. However, bodyweight gains were comparable between the control and high dose groups from week 3 onwards. No other adverse effects were reported. No treatment-related effects on organ weights, gross pathology and histopathological changes were observed.

No dermal or inhalation repeated dose studies on MIT alone were available.

Overall, in agreement with the DS, RAC considers that the consistent findings from the 90 day studies are not sufficiently serious for justify classification for repeated toxicity. **No classification for STOT-RE** is appropriate.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that MIT did not induce mutations in bacteria and mammalian cells and it did not increase the frequency of chromosomal aberrations in mammalian cells. It also gave negative results in *in vivo* study in mice where it did not increase the formation of micronuclei. Neither did it increase the unscheduled DNA synthesis in primary rat hepatocytes.

It was concluded by the DS that no classification for mutagenicity is warranted according to the CLP Regulation.

Comments received during public consultation

One manufacturer agreed with the conclusion that MIT is not mutagenic.

Assessment and comparison with the classification criteria

Six *in vitro* mutagenicity studies were described in the CLH Report. Negative results were reported in 2 bacterial mutagenicity studies, 2 gene mutation studies (HPRT) in Chinese hamster ovary cells (CHO) and 2 chromosome aberration studies (one in CHO cells and one in human lymphocyte cultures).

The 3 available *in vivo* studies on MIT gave negative results. In a micronucleus assay, the frequency of micronucleated polychromatic erythrocytes in the bone marrow of CD-1 mice was not increased by MIT. In a second study, a negative result was found using an alternative strain of mice. A slight decrease in the PCE/NCE ratio was noted in high dose females at 24 and 48 hours after treatment and in high dose males at 24 hours. The results of a rat liver unscheduled DNA synthesis assay were negative.

On the basis of the negative results obtained from the *in vitro* and in *vivo* mutagenicity studies, RAC agrees with the DS that **classification of MIT for mutagenicity is not warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No data were available.

Comments received during public consultation

One manufacturer agreed with the DS that MIT is not carcinogenic and that no classification is required.

The DS explained that carcinogenicity/chronic toxicity studies performed with the related substance C(M)IT/MIT were included in the first draft of the CLH report. However these studies were later removed from the report because ECHA commented that the studies performed with CMIT/MIT (3:1) are not relevant to the classification of MIT. No classification of MIT for carcinogenicity has been proposed by the DS since no data on MIT are available.

Assessment and comparison with the classification criteria

There are no available data on the chronic toxicity and carcinogenicity potential of MIT. Clear negative results from mutagenicity studies provide reassurance that no classification is appropriate for carcinogenicity. Furthermore, regarding the question of a non-genotoxic carcinogenic hazard, there were also no indications from the repeated dose studies to indicate that MIT may potentially be carcinogenic. **No classification of MIT is proposed as there are no data demonstrating a possible carcinogenic hazard**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS summarised three teratology studies. No developmental effects were observed either in rats or rabbits treated with MIT. The NOAEL for maternal toxicity in one study on rats was determined to be 20 mg/kg bw/day based on reduced body weight gain and reduced food consumption and developmental NOAEL 40 mg/kg bw/day. In the second developmental study in rats maternal NOAEL 33.4 mg/kg bw/day was derived, since at higher doses statistically significant and dose-dependent reduction in mean body weight gain (16 % at 75 mg/kg bw/day and 30 % at 50 mg/kg bw/day) and food consumption (12 % at 75 mg/kg bw/day and 16 % at 50 mg/kg bw/day) were observed during treatment. Developmental NOAEL in this study was 33.4 mg/kg bw/day since at maternally toxic doses increased incidence of anomaly (dilated cerebral ventricles) and incomplete ossification were observed.

In rabbits, maternal NOAEL 10 mg/kg bw/day was determined based on decreased defecation, dark red areas in the stomach, body weight loss and reduced mean food consumption and developmental NOAEL 30 mg/kg bw/day.

No effects on fertility and sexual function in rats were observed in a three-generation reproductive toxicity study in rats. Reduced body weight gain and reduced food intake were observed in parents and their offspring. Based on the results of the relevant reproductive toxicity studies, the highest dose tested 1000 ppm (69-93 mg/kg/day) was not toxic for reproduction. However, the DS reported the following test substance-related effects at 1000 ppm MIT:
(1) Decreased mean body weight gains in males and females during the first one-to-five weeks of each generation and during the middle and/or late parts of gestation and lactation; decreased mean body weights beginning at week 2 or 3 and continuing throughout the remainder of the generation (F0) or throughout the generation (F1).
(2) Decreased food consumption throughout each respective generation (males); Decreased food consumption throughout the pre-breeding period and during middle-to-late gestation and middle-to-late lactation (F0 females); Decreased food consumption throughout the pre-breeding period and gestation periods and during middle-to-late lactation (F1 females); decreased food efficiency during the first four or five weeks of the study (F0 only). This finding was most likely associated with decreased water consumption.
(3) Decreased mean offspring body weights in the latter part of both the F1 pre-weaning period (post-natal days 7-21) and the F2 pre-weaning period (post-natal days 14-21).

Based on these findings, the dose level of 200 ppm (15-22 mg/kg/day for the F0 pre-mating period and 19-26 mg/kg/day for the F1 pre-mating period) is considered a NOAEL for parental toxicity and for neonatal toxicity.

It was concluded by the DS that no classification for reproductive toxicity is warranted according to the CLP Regulation.

Comments received during public consultation

One manufacturer agreed that classification of MIT for reproductive toxicity is not required.

One MS commented that changes in sperm parameters were observed in a repeated dose study (reduced sperm motility and sperm heads). Although these changes were within the relevant historical control range, the MS requested that these findings are included in the discussion on reproductive toxicity (fertility).

A second MS requested the DS to check the consistency of data in the CLH report.

Assessment and comparison with the classification criteria

Sexual function and fertility

In a three-generation study, F_0 male and female rats were exposed to MIT in drinking water at doses of 0, 50 200 and 1000ppm from 70 days prior to pairing, then throughout mating, gestation and lactation of two litters (F_1 and F_2).

Water consumption decreased in males at all dose levels and in females of the F0 and F1 generations during the gestation and lactation in the 200 and 1000ppm groups. Decreased bodyweight and food consumption were also observed in the 1000ppm group. Decreased water consumption was observed in F2 females at 200 and 1000ppm during the pre-breeding period. On PND 7, 14 and 21, decreased bodyweights of F1 and F2 pups were noted. The effects on water consumption are considered to have been due to adverse taste or smell of the test substance. A delay in the mean day of balanopreputial separation and vaginal patency was observed in the 1000ppm group of P1 pups. However, the results were within the historical control data range.

In this study, there were no treatment-related mortalities, clinical signs of toxicity, or macroscopic abnormalities. No effects on reproductive performance parturition or spermatogenic endpoints were observed.

However, changes in sperm parameters were observed in males Wister rats in a 90 d oral toxicity study. The rats were administered 0, 7.52, 15 or 30 mg/kg MIT (50.7% MIT in water), by gavage. In the high dose group, sperm motility was reduced. There was also a dose-dependent reduction in the number of testicular sperm heads in treated animals. For both effects, the results were within the historical control data range. There were no histopathological changes, no reduction in epididymal sperm count and no changes in testis weight. In all treatment groups, a statistically significant increase in mean percent of morphologically abnormal sperm cells from the cauda epididymis was observed (0.67 (standard deviation 0.66), 2.2 (SD 1.29), 2.35 (SD 1.25) and 2.80% (SD 1.09) at 0, 7.5, 15 and 30 mg/kg MIT, respectively). The DS did not elaborate on what was meant by abnormal sperm cells (i.e. tails or heads). The DS indicated that 4.05% and 4.95% of sperm cells were morphologically abnormal in the control 28 day recovery and high dose recovery groups, respectively. Given this, and the very flat dose-response observed at 90 days, it therefore seems likely that the observed effect was a consequence of the concurrent control value rather than due to dosing with MIT. Historical control data from 2-generation studies performed in Wister rats by the same laboratory further support this conclusion. The mean value was 5.3%.

No comparable findings were reported in another 90 day oral toxicity study, in which rats (different strain) were administered MIT in the drinking water at levels of 0, 75, 250 or 1000 ppm (the top dose was approx. 66 mg/kg in males and 94 mg/kg in females.

There is no evidence that MIT causes adverse effects on sexual function or fertility. Although changes in sperm parameters were observed in one of the 90-day repeated dose studies, the magnitudes of the effects were small and the findings were not supported by changes in other parameters including testes weight and epididymal sperm count. Furthermore, the incidence of morphologically abnormal sperm cells in all dose groups was below the historical mean for control Wistar rats (F0 generation; 2-generation studies). The reduced sperm motility and dose-dependent reduction in the number of sperm heads was also within the historical control data range. RAC concludes that, at most, there may be small effects on some sperm parameters. However, MIT treatment did not produce any clear adverse effects on the male reproductive system in any of the available studies. Notably, no effects on sperm parameters were observed after exposure to higher concentrations of MIT in the multi-generation study. This finding provides further reassurance that the apparent changes in sperm parameters in one of the 90 day studies were not treatment-related. Therefore, RAC agrees with the DS that **no classification is justified for effects on sexual function and fertility**.

Developmental toxicity

Three developmental toxicity studies were available: two in rats and one in rabbits.

In the first study, female rats were administered 0, 5, 20 and 60/40 mg/kg bw/d MIT on gestation days (GD) 6-19. On GD 6-9, the majority of animals received 60 mg/kg bw/day. Since this dose exceeded the maximum tolerated dose, the high dose was lowered to 40mg/kg bw/d.

In the high dose group, three animals were found dead and two were euthanised in the moribund state. Clinical signs noted in these animals included rocking, lurching or swaying while ambulating, hypoactivity, rales, gasping, laboured respiration, decreased defecation, and red material around the nose, mouth and/or eyes. In the surviving females of the high dose group, the following clinical findings were observed: gasping, laboured respiration, red areas in the glandular portion of the stomach, dark red discolouration of the lungs, dark red areas in the lungs and/or lungs not fully collapsed and reductions in bodyweight gain and food consumption.

At 5 and 20mg/kg bw/d, there were no treatment-related effects on mean bodyweight, bodyweight gain, gravid uterine weight, food consumption and internal findings at necropsy.

There was no effect on the number of corpora lutea or implantations, number of resorptions, fetal bodyweight or sex ratio. No treatment-related external, visceral or skeletal malformations were observed in the fetuses.

In the second study, female rats were exposed by gavage to 0, 33.4, 49.8 and 75 mg MIT/kg bw/d on GD 6-15. No maternal deaths or clinical signs were observed. At 49.8 and 75 mg/kg bw/d, there was a significant and dose-dependent reduction in bodyweight gain of dams and a significant decrease in food consumption.

There were no significant differences in the number of corpora lutea, implantations, viable fetuses, embryonic deaths, fetal deaths, pre- and post-implantation loss, mean fetal bodyweights and placental weights.

The main effects observed in the study are tabulated below (Reference: Expert Witness Statement provided to RAC by CiToxLAB on behalf of Thor , 25 February 2016).

	Dose (mg/kg bw/day)			
	0	**33**	**50**	**75**
Visceral examination				
Dams	25	24	21	22[1]
Foetuses examined	169	162	146	154
Cerebral lateral ventricles dilated - foetusus affected (%) - litters affected (%)	 3 (1.8) 3/25 (12)	 8 (4.9) 6/24 (25)	 5 (3.4) 5/21 (24)	 19 (12) 14/22 (64)**
Skeletal examination				
Dams	25	24	21	22
Foetuses examined	169	166	147	157
Cervical vertebral bodies unossified - foetusus affected (%) - litters affected (%)	 90 (53) *	 96 (58) 23/24 (96)	 112 (76)** 21/21 (100)	 113 (72)** 21/22 (91)
Metatarsals unossified - foetuses affected (%) - litters affected (%)	 96 (57) *	 96 (58) 21/24 (88)	 94(64) 20/21 (95)	 122 (78)** 21/22 (95)

**- $p < 0,01$ Chi^2

A significant increase in the number of unossified cervical vertebral bodies was observed at 49.8 and 75 mg/kg (76% foetuses, 21/21 litters and 72% foetuses, 21/22 litters respectively). However, this study was performed in 1999 and hence the recording and reporting of data does not completely conform with today's protocol for an OECD 414 study. In the report of the 1999 study, the observation recorded was 'Cervical Vertebral Bodies – unossified/ one or more'. According to the statement from the test laboratory, the raw data shows that in most cases, all 7 cervical vertebrae were recorded as unossified in all groups, which is not biologically plausible. This observation is therefore likely to be an artefact of staining. According to standards applicable today, cases where the cervical vertebra bodies are missing or abnormal would be recorded in accordance with current standardised recording of foetal skeletal examinations (Makris et al. 2009). No such observations were made in this study. Additionally, although the incidence of the finding in controls appears to be high (53%), it was actually considered to be relatively low compared with the overall expected incidence. This may explain the statistical significance at the top two doses.

At 75 mg/kg, the number of visceral variations was found to have decreased significantly in the foetuses. However, at this dose, a significant increase in dilated cerebral ventricles was observed in foetuses.

Dilated cerebral ventricles are subjective observations based on the ventricle size that is considered normal by the observer. Furthermore, it is thought that the sectioning process can

[1] This value of 22 is given in the study report, contrary to the value of 21 given by the DS in the RCOM (response to comment number 7.

cause artefacts, which may resemble dilated cerebral ventricles. It is understood that if this study was conducted again today, this finding would only be recorded if the dilatation was deemed to be significant, and this would usually be observed in combination with oedema in other regions of the brain and head. In addition, the statistically significant increase in the number of litters affected at the top dose may be attributed to the relatively low incidence in concurrent controls. On this basis, the observations of dilated cerebral ventricles are considered to be artefacts and therefore not relevant for classification of MIT for developmental effects.

At 75mg/kg, the number of unossified metatarsals (78% fetuses, 21/22 litters) was reported to be significantly higher than the control value. The DS considered that the delay in ossification was probably due to the decreased bodyweight gain of the dams. However, as in the case of unossified cervical vertebral bodies, the reporting of unossified metatarsals in this study differs from how the findings would be recorded today. According to current standards, unossification of more than one of the metatarsals would be reported as a variation. In the 1999 study, only one foetus has 2 unossified metatarsals (in the mid dose group). All of the other foetuses had up to one unossified metatarsal. Therefore, the recorded observations were not variations according to the currently applicable standard. As with unossified cervical vertebral bodies, the incidence of unossified metatarsals in concurrent controls (57%) was considered to be relatively low compared with the overall expected incidence, which may explain the statistical significance at the top dose.

The DS considered that the significant differences in the incidence of supernumerary ribs between the control group and the 33.4 mg/kg bw/d group was not biologically significant.
In rabbits, MIT was administered at 0, 3, 10 and 30 mg/kg MIT on gestation days 6-28 and the animals were sacrificed on day 29. At the top dose, one dam aborted on day 25. Another dam was found dead on day 19. Treatment-related effects observed at this dose included decreased defecation, dark red areas in the stomach, mean bodyweight loss (GD6-9), and reduced mean food consumption. There was no evidence of developmental toxicity at doses up to and including 30mg/kg bw/d.

Overall, RAC is of the opinion that the results of these studies are not considered to support classification of MIT as a developmental toxicant. The observations of dilated cerebral ventricles in rats are considered likely to be artefacts of sectioning rather than true developmental effects. The statistically significant increase in the incidences of unossified cervical bodies and metatarsals in rats is most likely to be due to the relatively low incidence in controls compared to the overall expected incidence. Moreover, according to the test laboratory, none of these 3 reported observations in rats would be recorded under current guidelines (Makris et al. 2009). The results from the rabbit study did not raise a concern for developmental toxicity. Therefore RAC agrees with the DS that **no classification for reproductive toxicity is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

2-methylisothiazol-3(2H)-one (MIT) is not currently listed in Annex VI of CLP (Regulation (EC) 1272/2008). The DS proposed to classify the substance as Aquatic Acute 1 – H400 (M=10) and Aquatic Chronic 1 – H410 (M=1). The evaluation of the DS was based on the data provided by two different applicants (Rohm and Haas as well as Thor) submitted within the framework of the Biocidal Products Regulation.

Degradation

The available hydrolysis studies indicated that MIT is hydrolytically stable at all pHs tested. In the first key study (Rohm and Haas) carried out according to EPA 161-1 guideline and in compliance with GLP, no significant hydrolysis of MIT was observed at pH 5, 7 and 9 as the compound was stable for more than 720 hours at 25 °C. The second key study (Thor), performed according to OECD TG 111 and in compliance with GLP, was run at pH 4, 7 and 9 at 50°C for 5 days and showed that MIT is hydrolytically stable under acidic, neutral and alkaline conditions. No hydrolysis rate constants k and DT_{50} values could be calculated from these two studies as the substance was stable to hydrolysis at all pH conditions.

The photodegradation of MIT in water was studied in two studies performed according to US EPA 161-2 guideline and in compliance with GLP. In the first one (Rohm and Haas) conducted at 25°C and pH 7 under natural sunlight for 30 days, MIT was shown to be photolytically degraded at a moderate rate (half-life 11.1 days). Two major photodegradates were produced: 3-methyl-4-thiazolin-2-one and a mixture of N-methyl malonamic acid as primary component, with smaller quantities of N-methyl acetamide and N-methyl oxamic acid. In the second study the phototransformation in water was conducted at 25°C and pH 7 under artificial sunlight conditions. The half-life, extrapolated to natural sunlight under the chosen conditions, was 18.2 days. Three relevant transformation products were formed: UNK 8, slightly more apolar than MIT, UNK 4 and UNK 10, both more polar than the parent.

Three ready biodegradation studies are available showing that MIT is not readily biodegradable. In the first one, carried out according to OECD TG 301B (Modified Sturm Test), three different test solutions were tested (0.01, 0.03 and 0.1 mg/L). The biodegradation of the substance after 28 days of incubation was 48-56%. In the second study, performed according to OECD TG 301D (Closed Bottle Test), no biodegradation of MIT was observed within 28 days, even if toxic effects of the test substance on the inoculum at the actual test concentration of 10 mg/L could not be excluded and no explanation was given for the high oxygen demand in the inoculum control. In the last study, the biological degradation of the substance reached the maximum of 12-17% after 29 days in a 36-day DOC Die Away ready biodegradability test (OECD TG 301A).

Two aquatic biodegradation simulation studies (according to OECD TG 309) are available: in estuarine water, carried out at 20°C for 6 days with a test substance concentration of 22 and 112 µg/L (Guo I. *et al.*, 2007b) and in freshwater, carried out at 20±2°C for 56 days with a test substance concentration 2 and 97.5 µg/L (Thor). A supporting study (Thor - Hamwijk *et al.* 2007b) in seawater was carried out at 15±2°C for 56 days, with test concentration 1.5 and 87.5 µg/L according to OECD TG 309 (Hamwijk and Cremers 2007b). The results were recalculated to reflect an average EU outdoor temperature of 12°C for the freshwater compartment and 9°C for the marine environment. The primary degradation half-lives of MIT in estuarine water, at 20°C (12°C), were 1.38 (2.63) days for 22 µg/L and 1.24 (2.35) days for 112 µg/L (Guo *et al.*, 2007b); in freshwater the results showed that MIT was almost completely degraded (>95%) after 56 days, but no samples were taken between 0 and 7 days. Therefore, no conclusion could be drawn regarding the half-life of MIT or the formation of major metabolites. After 7 days only approximately 25% of radioactivity was still present as MIT. The data have been only considered as supportive for a rapid primary biodegradation of MIT in freshwater. The results of the supporting study in seawater were calculated only for the concentration of 87.5 µg/L: the primary degradation half-life at 15°C (9°C) was 3.6 (5.7) days. The major metabolite in the estuarine water study was identified as N-methyl malonamic acid, whereas in seawater metabolites were detected but not identified.

Two aerobic water/sediment simulation studies (Rohm and Haas, Thor) were carried out at 20°C for 30 days and 100-101 days, respectively, according to OECD TG 308. The results from laboratory studies have been recalculated to reflect an average EU outdoor temperature of 12°C. The half-lives for primary degradation of MIT in the water/sediment compartment (whole system) are very short: from a few hours (0.87 days) to a maximum of 4.17 days. Metabolites in the study by Rohm and Haas were identified as 2-(methylcarbamoyl) ethene sulfonic acid and 2-hydroxyethane sulfonic acid. In the study by Thor no metabolites could be identified.

Bioaccumulation

The experimental log Kow for MIT is -0.32 and was determined in a study at pH 7 and 20°C carried out according to OECD TG 117. This value was several orders of magnitude lower than the CLP trigger value of 4 intended to identify substances with a potential to bioaccumulate.

Aquatic toxicity

Regarding aquatic toxicity, the available studies on MIT are presented in the table below. In total there are three acute and two chronic aquatic toxicity tests on fish, three acute and two chronic toxicity tests on aquatic invertebrates and three toxicity tests on algae available.

Summary of relevant information on aquatic toxicity.

Method	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
OECD TG 203 US EPA 72-1 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	96-h LC_{50}	4.77	A7.4.1.1.a/01 (Rohm and Haas)
US EPA 72-1 US EPA OPPTS 850.1075 Freshwater	*Oncorhynchus mykiss*	Semi-Static mm	96-h LC_{50}	5.71	A7.4.1.1.a/02 (Thor)
OECD 203 US EPA OPPTS 850.1075 Marine water	*Cyprinodon variegates*	Flow-through mm	96-h LC_{50}	25.1	A7.4.3.2.b/01 (Rohm and Haas)
Long-term toxicity to fish					
OECD TG 210 US EPA OPPTS 850.1075 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	98-d NOEC (based on growth, wet weight)	2.38	A7.4.3.2.a/01 (Rohm and Haas)
OECD TG 210 US EPA OPPTS 850.1075 Freshwater	*Pimephales promelas*	Flow-through mm	33-d NOEC (survival)	2.1	A7.4.3.2/01 (Thor)
Short-term toxicity to aquatic invertebrates					
OECD TG 202 US EPA 72-2 Freshwater	*Daphnia magna*	Flow-through mm	48-h EC_{50}	0.998	A7.4.1.2.a/01 (Rohm and Haas)
US EPA 72-2 US EPA OPPTS 850.1010	*Daphnia magna*	Semi-Static m	48-h EC_{50}	1.68	A7.4.1.2/01 (Thor)

Method	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
US EPA 40 CFR 797.1300 Freshwater					
US EPA OPPTS 850.1035 Marine water	*Americamysis bahia*	Flow-through mm	96-h LC_{50}	1.81	A7.4.1.2.b/01 (Rohm and Haas)
Long-term toxicity to aquatic invertebrates					
OECD TG 211 US EPA OPPTS 850.1300 Freshwater	*Daphnia magna*	Flow-through m	21-d NOEC (based on dry weight)	0.0442	A7.4.3.4/01 (Rohm and Haas)
OECD TG 211 US EPA OPPTS 850.1300 Freshwater	*Daphnia magna*	Flow-through m	21-d NOEC (based on dry weight)	0.55	A7.4.3.4/01 (Thor)
Toxicity to algae					
OECD TG 201 US EPA FIFRA 122-2 EEC.3 Freshwater	*Pseudokirchneriella subcapitata*	120h Static imc	24-h E_rC_{50} 24-h E_rC_{10}	0.102 0.062	A7.4.1.3.b/01 (Rohm and Haas)
US EPA FIFRA 123-2 US EPA OPPTS 850.5400 Freshwater	*Pseudokirchneriella subcapitata*	96h Static imc	24-h E_rC_{50} **24-h E_rC_{10}**	0.114 **0.024**	A7.4.1.3-01 (Thor)
US EPA FIFRA 123-2 Marine water	*Skeletonema costatum*	120h Static imc	**24-h E_rC_{50}** 24-h E_rC_{10}	**0.0695** 0.044	A7.4.1.3.b/01 (Rohm and Haas)

m – measured concentration
mm – mean measured concentration
nom – nominal concentration
imc – initial measured concentration
Key endpoints used in acute and long-term hazard classification are highlighted in bold.

The freshwater acute and chronic toxicity values for fish were in the same concentration range. The marine species was shown to be less sensitive.

The long-term study on *Daphnia* with a NOEC value (0.0442 mg/L) was of the same order of magnitude as the NOEC obtained for the freshwater alga species *Pseudokierchneriella subcapitata* (0.024 mg/L). The $NOEC_{growth}$ for *Daphnia* was based on significant effects on dry weight. It should be noted that growth is an optional test parameter according to OECD TG 211. Although the guideline is designed principally to assess effects on reproduction, other effects might allow a statistical analysis. Indeed growth measurements could provide information on possible sublethal effects useful in addition to reproduction measures alone. The available information did not show a clear dose-response relationship.

Regarding toxicity to algae, the DS provided two toxicity studies on *Pseudokirchneriella subcapitata* and a toxicity study on the saltwater diatom *Skeletonema costatum*. All the studies are static tests and the derived endpoints were based on initial measured concentrations. The concentration of the test substance was not maintained at >80% of nominal concentrations, due to fast biodegradation of MIT in the presence of algae, and the exponential increase in cell density

in the controls was not maintained after 72 h. This can be attributed to the peculiar behaviour of the substance in the presence of algae by means that the degradation of MIT depends on the algal concentration (*i.e.* the concentration dependency can be attributed to the role of algae in the degradation of MIT). MIT is rapidly taken up by the algae, and inhibits enzymes by binding to the thiol-groups of the proteins. A consequence of this binding is cleaving of the isothiazolone ring and further degradation. This means that the inhibitory effect on algae will also result in a degradation of MIT by algae. At higher test concentrations toxic to algae, growth of algae is inhibited which in turn slows down the degradation of MIT by algae. The mode of action of MIT implies that the sensitivity of the test is affected by the cell density. Therefore, the removal of MIT from the test system is rapid and a NOEC based on geometric mean concentration does not take into account the interaction between algal density and biodegradation of MIT. For this reason, the 24 hour E_rC_{10} based on initial measured concentrations was used as endpoint.

Moreover, acute toxicity data for the three major metabolites of MIT (N-methyl malonamic acid, N-methyl acetamide and malonamic acid) are available on all three trophic levels (table below). For algae also chronic endpoints are available.

Summary of relevant information on aquatic toxicity of MIT metabolites

Method	Test organism	Conditions/Metabolite	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static nom N-methyl malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/01 (Rohm and Haas)
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm N-methyl acetamide	96-h LC_{50}	>694	A7.4.1.1.c/02 (Rohm and Haas)
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/03 (Rohm and Haas)
Short-term toxicity to aquatic invertebrates					
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static nom N-methyl malonamic acid	48-h EC_{50}	> 1000	A7.4.1.2.c/01 (Rohm and Haas)
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static mm N-methyl acetamide	48-h EC_{50}	> 863	A7.4.1.2.c/02 (Thor)
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static nom malonamic acid	48-h EC_{50}	> 1000	A7.4.1.2.c/03 (Rohm and Haas)
Toxicity to algae					
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96h-Static imc N-methyl malonamic acid	72-h E_rC_{50} 96-h E_rC_{50} 72-h	97 128 36 36	A7.4.1.3c/01 (Rohm and Haas)

Method	Test organism	Conditions/Metabolite	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static nom N-methyl malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/01 (Rohm and Haas)
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm N-methyl acetamide	96-h LC_{50}	>694	A7.4.1.1.c/02 (Rohm and Haas)
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/03 (Rohm and Haas)
Short-term toxicity to aquatic invertebrates					
			NOE_rC 96-h NOE_rC		
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96h-Static imc N-methyl acetamide	72-h E_rC_{50} 96-h E_rC_{50} 72-h NOE_rC 96-h NOE_rC	5.8 5.7 0.51 0.51	A7.4.1.3c/02 (Rohm and Haas)
OECD TG 201 US EPA OPPTS 850.5400 US EPA TSCA 797.1050 US EPA FIFRA 122-2 and 123-2	*Pseudokirchneriella subcapitata*	96h-Static imc Malonamic acid	72-h ErC50 96-h ErC50 72-h NOErC 96-h NOErC	> 1080 > 1080 1080 519	A7.4.1.3c/03 (Rohm and Haas)
mm – mean measured concentration nom – nominal concentration imc – initial measured concentration					

Short-term toxicity tests to fish and aquatic invertebrates indicated that all three metabolites were practically non-toxic for these trophic levels. The studies on algae indicated that all three metabolites were less toxic (1 to 4 orders of magnitude lower) than the parent MIT. However, an algae NOEC value of 0.51 mg/L for NMA showed that this metabolite was toxic to algae.

Comments received during public consultation

Two MSCA and three companies commented on the proposed environmental classification.

Regarding aquatic toxicity, one MSCA supported the use of the 24h-ErC_{10} endpoint on algae for classification and proposed some modifications to harmonise some sections of the CLH report, which were agreed by the DS. According to another MSCA, it was not clear if the algal endpoint reflected the validity criteria of exponential growth in controls at 48h and therefore proposed that the classification should be based on endpoints which reflect the usual period of exponential

growth (72h or 96h). The DS replied that the exponential growth in the control was demonstrated for 72h, including the first 24h. Moreover, the DS stated that the approach to deviate from standard 72h or 96h was in line with the CLH proposal for the substance C(M)IT/MIT (CAS n. 55965-84-9), which is also an isothiazolinone and has a similar mode of action on algae as MIT.

One company did not agree to deviate from CLP by using the algal endpoint based on 24h values for classification. Moreover, the company did not support the chronic M-factor of 1 because the degradation products degrade rapidly and are less toxic than the parent compound. Finally, the company suggested to use the NOErC from the marine algae study, which will lead to no chronic M-Factor.

The DS in his reply emphasised that the choice of the EC_{10} rather than NOEC as endpoint for classification was statistically more robust because it was derived from the dose-response curve and was shown to be less affected by variability in the control performance which tends to be higher during the first 24h of tests with algae. Moreover, the decision to consider the substance as not rapidly degradable was based on the fact that not all the metabolites formed at >10% have been successfully identified. Therefore, the choice of a chronic M-Factor of 1 was considered justified in a weight of evidence approach. Finally, the DS referred to another CLH proposal for the substance C(M)IT/MIT (CAS n. 55965-84-9) presenting an additional degradation study carried out with MIT.

Another company suggested to classify as H400 and H411 with a M factor=1 because the algal test proposed was not considered suitable for classification. According to the company, the validity criteria of the normal duration of the test (at least 72h) has not been taken in consideration and the validity criteria for the control performance must also be taken into account. In addition, in view of the rapid dissipation of the substance from the test media, it is expected that algae will not be affected in the long term. The DS replied that the validity criteria of the control performance were met also for the first 24h and, in view of the specific behaviour of the substance in the presence of algae, daily analytical measurements should preferably be performed.

Assessment and comparison with the classification criteria

Degradation

MIT is stable to hydrolysis at all pH values tested. Regarding photodegradation in water the reported half-life was 11.1-18.2 days. The ready biodegradation studies showed that MIT was not readily biodegradable. The primary biodegradation half-lives of MIT in the aquatic environment were very short, ranging from a couple of hours to a maximum of 4.17 days. However, not all metabolites detected at greater than 10% were definitively identified. The lack of identity information provided in the CLH report for all transformation products did not allow a conclusion on their classification as hazardous to the aquatic environment. In addition, one of the known transformation products (N-methyl acetamide) is classifiable as Aquatic Chronic 3, based on an algae NOEC value of 0.51 mg/l and its rapid degradability. Finally, in the CLH report for C(M)IT/MIT, an additional degradation study in seawater carried out on MIT resulted in a DT_{50} (primary degradation) of 29.7 days at 9 °C.

Based on this information, MIT was considered not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

The experimental log Kow of MIT is -0.32, this is orders of magnitude lower than the trigger value of 4 in the CLP Regulation for substances showing a potential for bioaccumulation.

Aquatic toxicity

Acute toxicity data are available for all three trophic levels. The most acutely sensitive trophic group was algae with a 24-h ErC_{50} value for *Skeletonema costatum* of 0.0695 mg/L. This acute endpoint is in the range of $0.01 < L(E)C_{50} \leq 0.1$ mg/L.
Chronic toxicity data are available for all three trophic levels. The most acutely sensitive trophic group was algae with a 24-h ErC_{10} value for *Skeletonema costatum* of 0.024 mg/L. This chronic endpoint is in the range of $0.01 < NOEC/ECx \leq 0.1$ mg/L.

Conclusion

MIT is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation potential. The lowest acute toxicity value falls in the range $0.01 < L(E)C_{50} \leq 0.1$ mg/L and the lowest chronic toxicity value lies in the toxicity range of $0.01 < NOEC/ECx \leq 0.1$ mg/L.
RAC concluded that MIT fulfils the CLP criteria for classification as **Aquatic Acute 1 - H400** with an **M-factor of 10** and **Aquatic Chronic 1 – H410** with an **M-factor of 1.**

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).