Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride

EC Number: 247-499-3
CAS Number: 26172-54-3

CLH-O-0000007341-81-01/F

Adopted

14 September 2023

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CLH-O-0000007341-81-01/F

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

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride**

EC Number: **247-499-3**

CAS Number: **26172-54-3**

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

Co-Rapporteur, appointed by RAC: **Irina Karadjova**

Administrative information on the opinion

Slovenia has submitted on **29 September 2022** 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 November 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 January 2023**.

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

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

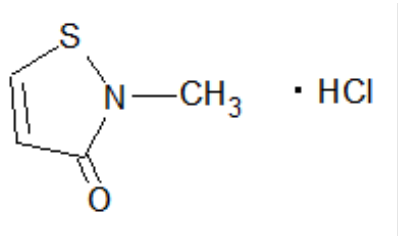
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride	247-499-3	26172-54-3	Acute Tox. 3 Skin Corr. 1A Eye Dam. 1 Skin Sens. 1A Acute Aquatic 1 Acute Chronic 1	H301 H314 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H301 H314 H317 H410	EUH071	oral: ATE = 175 mg/kg bw dermal: Skin. Sens 1A; H317: C ≥ 0.0015% M=1 M=1	
RAC opinion	TBD	2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride	247-499-3	26172-54-3	Acute Tox. 3 Acute Tox. 3 Acute Tox. 2 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1A Acute Aquatic 1 Acute Chronic 1	H301 H311 H330 H314 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H301 H311 H330 H314 H317 H410	EUH071	oral: ATE = 180 mg/kg bw dermal: ATE = 320 mg/kg bw inhalation: ATE = 0.15 mg/L (dusts or mists) Skin. Sens 1A; H317: C ≥ 0.0015% M=10 M=1	
Resulting Annex VI entry if agreed by COM	TBD	2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride	247-499-3	26172-54-3	Acute Tox. 3 Acute Tox. 3 Acute Tox. 2 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1A Acute Aquatic 1 Acute Chronic 1	H301 H311 H330 H314 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H301 H311 H330 H314 H317 H410	EUH071	oral: ATE = 180 mg/kg bw dermal: ATE = 320 mg/kg bw inhalation: ATE = 0.15 mg/L (dusts or mists) Skin. Sens 1A; H317: C ≥ 0.0015% M=10 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride (MIT·HCl) is a new biocidal active substance (under EU No 528/2012), not previously discussed and/or agreed by the Technical Committee on Classification and Labelling (Dir. 67/548/EEC) and/or RAC (CLP Regulation).



2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride MIT·HCl: EC number: 247-499-3; CAS number: 26172-54-3

Uses

PT 6: Preservatives for products during storage

PT 6.7: Mineral slurries and other matrices

Active substance is intended to be used as a preservative in aqueous solutions in life science, specifically in reagents for scientific research and development in life science sector and in the production of biomolecules. The preserved products may be biopharmaceuticals, medical devices, nutraceuticals, or tools for forensic sciences or molecular biology including both large industrial installations and small and medium laboratories.

- used as a preservative in different water-based solutions like antibody-, protein solutions used for protein isolation and separation and isolation steps in immunoassays as well as nucleic acid preparations
- used as a preservative in buffer solutions for medical and scientific research market in a controlled laboratory environment
- used as a preservative in different filtration membranes and filters
- used as a preservative in chromatography resins, which are used to purify proteins and nucleic acids in a variety of life sciences applications, such as R&D and manufacturing of human therapeutics, vaccines, gene therapy medicines and somatic-cell therapy medicines

For several hazard classes, the proposal was based on read across from the already classified 2-methyl-2*H*-isothiazol-3-one (MIT; EINECS 220-239-6; CAS No 2682-20-4) (free base) to MIT·HCl. The read across was justified according to the ECHA Read-Across Assessment Framework (RAAF) Analogue Scenario 1, (bio)transformation to common compound(s). The main data source were data on MIT. Also data from related REACH registration dossiers were reviewed.

As described by the dossier submitter in the CLH report, the pure/non aqueous MIT-HCl is only present in solid state immediately after manufacturing. Once MIT-HCl is in an aqueous solution, it dissociates into MIT and HCl. Chloride and hydrogen ions originating from MIT·HCl do not represent a biologically significant amount and do not contribute to the overall efficacy at the used MIT·HCl concentrations. MIT in aqueous solution originating from MIT-HCl is chemically indistinguishable from MIT in aqueous solution originating from MIT. In an aqueous environment the nitrogen atom in MIT can exist as a protonated ion (conjugated acid) or an unprotonated free base with hydrogen from surrounding water molecules being used as the source of protons. The

extent of protonation depends on the pH of the local environment. The pH in solutions, where MIT is active, is from 6-8. The dominant form for MIT will be the unprotonated form at pH 6-8. Therefore, RAC considers that it is chemically equivalent to MIT.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

MIT·HCl does not fulfil the criteria of the screening procedure as there are chemical groups associated with explosive properties in the molecular structure.

The DSC screening test was performed because of the presence of N, O and Cl in the MIT·HCl molecule which showed a total decomposition energy < 500 J/g and the onset of exothermic decomposition < 500 °C.

The DS concluded that MIT·HCl **did not warrant classification** as explosive considering CLP I.2.1.4.3(c).

Flammable solids

The DS concluded that the criteria for classification as a flammable solid are not met. A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. MIT·HCl melted (melting temperature between 165.0 °C to 170.0 °C) but did not ignite, so the DS proposed **no classification**.

Self-reactive substances

Data available from UN H.4 Heat Accumulation Storage Test showed that 37.9 h are taken for MIT·HCl to reach a temperature 2 °C below the oven temperature. Over the following 168 h (7 days) the test item reached a maximum temperature of 75.3 °C (SADT > 75 °C for a 50 kg package). The DS concluded that MIT·HCl **does not warrant classification** as a self-reactive substance.

Pyrophoric solids

The DS predicted negative results from the procedure compatible with Method A13 Pyrophoric Properties of Solids and Liquids of Commission Regulation (EC) No 440/2008 of 30 May 2008 based on the experience in handling and use of the test item during testing. In addition, the DSC under 30 bar air pressure shows no exothermic behaviour up to 100°C, so the DS concluded **no classification** for pyrophoric solids.

Self-heating substances

Assessment of MIT·HCl for self-heating properties is based on negative results from the UN Test N.4, 2009, performed with the test item in 25 mm (at 140 °C and at 100 °C) and 100 mm cube (at 100 °C) samples. As the test results indicated that MIT·HCl is not a self-heating substance, the DS proposed **no classification**.

Substances which in contact with water emit flammable gases

The DS proposed no classification based on experience with use and handling predicting negative results the procedure designed to be compatible with method EC A.12 flammability (contact with water) of solids and liquids.

The DS proposed that MIT·HCl **does not warrant classification** as it does not react with water giving flammable gases.

Oxidising solids

The substance MIT·HCl does not contain functional groups which are associated with oxidising properties. Therefore, the DS proposed **no classification** based on screening criteria.

Corrosive to metals

DS noted that test C.1 (UN Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria) is not applicable as the test material is a solid, it has a high melting point of 165-170 °C therefore will not become a liquid in transportation.

The DS proposed **no classification** for hazard corrosive to metals.

Comments received during consultation

No comments concerning physical hazards were received.

Assessment and comparison with the classification criteria

Explosives

The substance does not contain any chemical groups that are indicative of explosive properties. A negative EC A.14 study is available as supportive evidence. RAC agrees with the DS that **no classification is warranted** as the screening criteria are met (CLP I.2.1.4.3).

Flammable solids

The substance melted (melting temperature between 165.0 °C to 170.0 °C.) but did not ignite. RAC agrees that **no classification is warranted as flammable solid** according to CLP I.2.7.2).

Self-reactive substances

Experimental data available from a UN H.4 Heat Accumulation Storage Test showed that 37.9 h are taken for MIT to reach a temperature 2°C below the oven temperature. Over the following 168 h (7 days), the test item reached a maximum temperature of 75.3 °C (SADT > 75 °C for a 50 kg package). RAC agrees with the DS to conclude that MIT·HCl **does not warrant classification as a self-reactive substance** (CLP I.2.8.2.1(d)).

Pyrophoric solids

RAC agrees with DS that, based on experience in handling and use, **no classification of MIT·HCl for pyrophoric solids is warranted** (CLP I.2.10.4.1). This is supported by additional data indicating the DSC under 30 bar air, where MIT·HCl showed no exothermic behaviour up to 100 °C.

Self-heating substances

RAC agrees with DS that based on negative results from the UN Test N.4, 2009, performed in 25 mm (at 140 °C and at 100 °C) and 100 mm cube (at 100 °C) samples, MIT·HCl **does not warrant classification as a self-heating substance** (CLP I.2.11.2.1(a and b)).

Substances which in contact with water emit flammable gases

RAC agrees with the DS's on the conclusion for **no classification of MIT·HCl based on experience with handling, use, and manufacture** (CLP I.2.12.4.1).

Oxidising solids

The substance MIT·HCl does not contain functional groups which are associated with oxidising properties. RAC agrees with DS on the conclusion for **no classification based on these screening criteria** (CLP I.2.14.4.1).

Corrosive to metals

RAC considers that although MIT·HCl is a solid with a high melting point of 165-170 °C, it contains chlorine and therefore warrants assessment for corrosivity to metals. However, following the screening criteria in the CLP guidance (2.16.4.1) and that MIT·HCl has a melting point in excess of 55 °C, RAC agrees with the DS that **no classification for corrosivity to metals is warranted**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

One acute oral toxicity study in the rat was available for MIT·HCl (acute up-down study, OECD 425, GLP). The rest of the studies available, two acute dermal studies and three acute inhalation studies (all in the rat) were performed with MIT. No data for these two exposure routes were available for MIT·HCl. The biocidal active substance dossier (under EU No 528/2012) had proposed a waiver based of corrosivity, according to the DS in line with the BPR data requirements.

The DS had included the data available for MIT following the accordance check and in response to a request from ECHA. However, they noted that differences in pH between MIT and MIT·HCl (which is more acidic) may contribute to the severity of local effects, and hence also to greater acute dermal and inhalation toxicity. Consequently, the DS felt that read-across from studies with MIT for this endpoint may underestimate acute dermal and inhalation toxicity, and may not therefore not be appropriate.

Oral

In the up-down oral acute toxicity study in female Wistar rats (Anonymous 10, 2018), the test article was dispersed in purified water and administered at a dose volume of 10 mL/kg. Individual rats were dosed sequentially at the following dose levels until one of the stopping criteria (as defined in OECD 425) was met: 175, 550, 175, 550, 175 and 55 mg/kg bw.

One animal was tested at 55 mg/kg bw MIT·HCl, no clinical signs were observed.

Three animals were tested at 175 mg/kg bw. Of those, piloerection was observed in 2/3, dyspnoea in 2/3, hunched posture in 2/3, ataxia in 2/3 and death in 1/3 (found dead on day 2). In the first animal, piloerection was noted at 1-4 h after dosing, and dyspnoea at 2-3 h after dosing; in second animal there were no clinical signs, and the third animal showed signs of piloerection, dyspnoea and hunched posture at 2-4 h after dosing, ataxia at 3-4 h after dosing, and was found dead the second day of dosing.

At 550 mg/kg bw, two animals were tested, of those 1/2 presented piloerection, 1/2 decreased activity, 2/2 dyspnoea, 2/2 prone posture, 2/2 hypothermia, 2/2 ptosis, 2/2 tonic convulsion and 2/2 death (humanely killed 4 h after dosing). The first animal displayed piloerection at 1-6.5 h after dosing, decreased activity at 2-4 h, dyspnoea at 2-6.5 h after dosing, and signs of ptosis, prone posture and hypothermia at 6.5 h after dosing when it was humanely killed; the second

animal showed signs of prone posture and ptosis immediately after dosing, dyspnoea at 15 minutes, and hypothermia and tonic convulsions 1 hour after dosing when it was humanely killed.

All surviving rats achieved body weight gains during the first and second weeks of the study.

Surviving animals were killed on day 15 and all animals subsequently underwent a full necropsy. No macroscopic changes were noted at necropsy, except for pale lungs, red and thick fundus region of the stomach, gelatinous appearance of the mucosal surface of the fundus region of the stomach and small caecum, which were noted in the animal dosed at 550 mg/kg bw that was killed *in extremis* 4 h after dosing. LD_{50} was established at 175 mg/kg bw MIT·HCl.

The DS ***proposed to classify MIT·HCl for oral acute toxicity: Acute Tox. 3; H301 'Toxic if swallowed'; ATE = 175 mg/kg bw***.

Dermal

In the first dermal acute toxicity study (Anonymous 14, 1999), Crl:CD BR rats were exposed to MIT (Kordek™ 573T, 53.2 % MIT) at the dose levels of 100, 200, 400 mg/kg bw for males and females, and at 300 mg/kg for males. In the second one (Anonymous 23), Wistar rats were exposed to MIT (Acticide SR 3267, 49.0 % MIT) at the dose level of 4 082 mg/kg bw (corresponding to 2 000 mg/kg bw MIT).

Several animals in the 200-400 mg/kg bw dose groups were found dead at 24 or 48 h. Clinical signs were noted beginning on day 1 and included: red material around muzzle, red material around eyes, scant and/or no faeces, passiveness, laboured breathing, and ataxia. Surviving rats recovered from these signs by day 5. Body weight gain in surviving rats was decreased (29-48 %) among both sexes at 200 mg/kg bw and above when compared to historical control values. Skin effects were observed in both sexes at all levels beginning on Day 1 and continuing to Day 14. These effects included blanching, edema, darkened areas, eschar, sloughing, scabbed areas and desiccation. The LD_{50} was 242 mg/kg bw MIT. Clinical signs related to possible narcotic effects were passiveness and ataxia. Their prevalence is shown in the table below.

	100 mg/kg bw	**200 mg/kg bw**	**300 mg/kg bw**	**400 mg/kg bw**
Passiveness				
male	0 %	2/6 (33 %) 1 hour after dosing	3/6 (50 %) 1 hour after dosing	1/6 (17 %) 1 and 2 hours after dosing
female	2/6 (33 %) 1 hour after dosing	4/6 (67 %) 1 hour after dosing	not tested	1/6 (17 %) 1 hour after dosing
Ataxia				
male	0 %	0 %	1/6 (17 %) 1 hour after dosing	1/6 (17 %) 1 hour after dosing
female	0 %	3/6 (50 %) 1 hour after dosing	not tested	1/6 (17 %) 1 hour after dosing

In the second dermal acute toxicity study (Anonymous 23), no deaths were recorded and the LD_{50} was > 2 000 mg/kg bw MIT. Clinical signs included severe erythema, very slight/ slight oedema, scabbing, eschar and body weight stasis in females.

The DS proposed ***no classification*** for MIT·HCl although MIT is classified in Category 3, H311, because they considered the relevance of the studies with MIT to MIT·HCl questionable.

Inhalation

Three nose-only acute rat inhalation studies for MIT were available (all OECD 403, GLP).

In the first one (Anonymous 11, 1995) with RH-573 Technical (97.8 % MIT), groups of rats (6/sex) were exposed for 4 h at levels of 0.012, 0.046, 0.15, 1.07, 2.09 mg/L air (vapour, MMAD: 3.1-5.3 µm; GSD: 2.0-2.4; respirable fraction 33.5-54 %).

In the second study (Anonymous 12, 2001; 2002), groups of rats (5/sex) were exposed for 4 h with Kordek™ 573T (53.2 % MIT), exposure levels were 0.15, 0.25, 0.47 and 0.68 mg/L MIT (vapour, MMAD: 2.2-2.5 µm; GSD: 1.7-1.9).

In the third study (Anonymous 31, 2000), groups of rats (5/sex) were exposed for 4 h with Acticide SR 3267 (49.8 % MIT), exposures were 0, 0.086, 0.173, 0.327 mg/L Acticide SR 3267, corresponding to 0, 0.042, 0.086, 0.163 mg/L MIT.

Mortalities were observed in all three studies, starting at the dose level 0.25 mg/L.

In Anonymous 11 (1995), most of the deaths occurred during the exposure and were considered to be due to the exposure of test material. The clinical signs seen in some, yet not all groups, included gasping, rales, labored breathing, respiratory noise, salivation, red-stained eyes and muzzle, nasal exudate, passiveness, and ataxia. Ataxia was recorded in 1/6 females (17 %) exposed to 2.09 mg/L at 3 h post exposure. Passiveness was recorded in 4/6 males (67 %) exposed to 0.15 mg/L at 3 h and at first day post exposure. There were no exposure-related effects on body weight gain of survivors. Necropsies revealed that animals in all the groups (either found dead or surviving) showed signs of slight to severe redness in all lobes of the lung. Scattered incidences of red pinpoint foci on the lungs and gas-filled stomachs were also observed. These necropsy observations were consistent with the clinical signs of respiratory irritation.

In Anonymous 12 (2001, 2002), Clinical signs were noted in both sexes at all exposure levels and included respiratory noise, gasping, rales, labored breathing, salivation, ataxia/abnormal gait, prostration, passiveness, scant and/or no feces, red material around eyes and/or muzzle, clear discharge from the nose, wet-matted fur around the muzzle, arched back and/or unkempt.

Deaths occurred at 0.25 mg/L (2M, 3F), 0.47 mg/L (1M, 3F) and 0.68 mg/L (5M, 4F) and during exposure or within 24 h. Clinical signs related to possible narcotic effects where passiveness, salivation, prostration, ataxia/abnormal gait and arched back. Percentage of animals affected during exposures are presented in the table below, for 5 animals per sex tested. Necropsy of the decedents revealed pale and/or reddened lungs, distended intestines and/or wet muzzle. Necropsy of the survivors revealed no gross changes. Females exposed to 0.25 mg/L and above had reduced (25-39 %) body weight gain during the 14-day observation period compared to historical controls. There was no effect on body weight in surviving males during the 14-day observation period.

Dosage, mg/L						
	0.15	**0.25**	**0.47**		**0.68**	
Passiveness			Percent animals affected	Time of exposure	Percent animals affected	Time of exposure
male	0 %	0 %	20 %	0-3.5 h	20 % 33 % 50 %	0-3.5 h 1 day 2 days
female	0 %	0 %	20 % 40 % 33 % 50 %	0 h 3.5 h 1 day 2 days	20 %	0 h
Salivation						
male	0 %	0 %	20 %	0-3.5 h		
female	0 %	0 %	20 %	0 h		
Prostration						
male	0 %	0 %			20 %	0-3.5 h
female	0 %	0 %	20 %	0 h		
Ataxia						
male	0 %	0 %	40 % 20 % 33 % 50 %	0 h 3.5 h 1 day 2 days		
female	0 %	0 %				
Abnormal gait						
male	0 %	0 %			20 % 50 %	3.5 h 1 day
female	0 %	0 %				
Arched back						
male	0 %	0 %				
female	0 %	0 %	50 %	2-5 days		

In Anonymous 31 (2000), the clinical signs included dyspnoea, cyanosis, laboured/increased breathing, hypoactivity, tremor, incoordination, squatting, piloerection and red ocular/nasal discharge. Animals exposed to 0.086 mg/L exhibited slight to moderate activity decrease, squatting position, piloerection, respiration rate increase and reddish discharge around the nose in the first hour after treatment. Animals recovered in the second hour after treatment. At 0.173 mg/L, dyspnoea and laboured breathing occurred in two male rats and one female (second hour of observation). The female animal died (3.5 hour) showing severe dyspnoea and laboured breathing. One male animal was found dead one day after the inhalation exposure. Before dying the animal showed moderate activity decrease, squatting position, cyanosis, piloerection, severe dyspnoea, noisy respiration and reddish discharge around the nose, tremor and incoordination. Survivors showed activity decrease, squatting position, piloerection, incoordination, tremor, dyspnoea, noisy respiration and reddish discharge around the nose from the first hour after the inhalation treatment. Animals recovered between the second and third day of the observation period. In animals exposed to 0.327 mg/L dyspnoea and laboured breathing occurred from 1.5 hour of the inhalation exposure. Three females died on the day of exposure showing severe dyspnoea and laboured breathing. One female was found dead one on Day 1. Survivors showed similar symptoms to those exposed to 0.086 mg/L and became symptom-free on the third day

of the observation period. After symptoms subsided the animal's behavior and general state during the remaining period of observation was normal in all dose groups.

The combined male and female LC_{50}-values were 0.11 mg/L MIT (Anonymous 11, 1995), 0.19 mg/L MIT (Anonymous 12 & 13 2001, 2002) and 0.134 mg/L MIT (Anonymous 31, 2000).

The DS proposed no classification for MIT·HCl although MIT is classified in Category 2., H330, because they considered the relevance of the studies with MIT to MIT·HCl questionable.

Comments received during consultation

One MSCA commented the proposal. They agreed with acute oral toxicity and the proposal to classify as Acute Tox. 3; H301 with an ATE of 175 mg/kg bw. They noted that also for MIT a classification with Acute Tox. 3 (H301) was derived by RAC (2016), and that it seems that the additional HCl included in MIT·HCl did not induce a substantially higher acute toxicity.

As for inhalation and dermal toxicity, they did not express their support or opposition explicitly. It was implied that they may not have agreed with the proposal to not classify, although they mentioned that it is agreed that the local potency of MIT·HCl might be higher than that of MIT. They however mentioned that the oral LD_{50} values of MIT and MIT·HCl are comparable and that data from Kathon™ (CMIT/MIT) could also be considered, although its classification as Skin Corr. 1C at concentrations ≥ 0.6 % indicates a lower local potency. They added that Kathon™ has a harmonised classification as Acute Tox. 2, H330 and Acute Tox. 2, H310.

Assessment and comparison with the classification criteria

Oral acute toxicity

In the acute oral up-down study study (Anonymous 10, 2018), the LD_{50} was 175 mg/kg bw in female rats. According to the CLP criteria, acute toxicity Category 3 applies for oral exposure (mg/kg bw) when 50 < ATE ≤ 300.

The ATE is based on the LD_{50} value of 175 mg/kg bw, rounded to 180 mg/kg bw.

Therefore, RAC agrees with the DS that **classification as Acute Tox. 3; H301 is warranted**, with an **ATE = 180 mg/kg bw** based on available data.

Dermal acute toxicity

No data were available for MIT·HCl, but there were two acute dermal studies (OECD 402, GLP) available for MIT. The resulting LD_{50} were very different: 242 mg/kg bw in Anonymous 14 (1999) and > 2000 mg/kg bw in Anonymous 23 (2000). RAC still agrees with the conclusion made in the RAC opinion for MIT adopted in March 2016 (CLH-O-0000001412-86-105/F): "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." No further data are available since this conclusion.

According to the CLP criteria, acute toxicity Category 3 applies for dermal exposure (mg/kg bw), when 200 < ATE ≤ 1 000.

However, the DS proposed to not classify MIT·HCl based on read-across from MIT, as they considered the relevance of the study with MIT to MIT·HCl questionable. RAC agrees with the DS in that the differences in pH between MIT and MIT·HCl (MIT·HCl being more acidic) may contribute to the severity of local effects, and hence also to greater acute dermal and inhalation toxicity., RAC considers that when classification is based on read-across from MIT, at minimum Category 3 would apply.

The available MIT·HCl study for acute oral toxicity (Anonymous 10, 2018) did not reveal a considerable difference when compared to the MIT acute oral toxicity studies. The LD_{50} for MIT·HCl was 175 mg/kg bw (Wistar females), while the established LD_{50} values for MIT in rats were 120 mg/kg bw (Crl:CD®BR females), 183 mg/kg bw (Crl:CD®BR females) and 247 mg MIT/kg bw (Wistar females). Also for skin corrosion, there is one *in vitro* study available for MIT·HCl and a similar *in vitro* study for MIT. While MIT·HCl appeared somewhat more corrosive than MIT (MIT·HCl optional Cat. 1A vs. MIT Cat. 1B/1C), considering the uncertainties involved, the difference is not considered major by RAC. Both are clearly skin corrosive substances.

As mentioned in the public consultation comment, also CMIT/MIT (3:1) has a dermal Acute Tox 2. classification (in addition to inhalation Acute Tox. 2 and oral Acute tox. 3). For skin corrosion, CMIT/MIT has Skin Corr. 1C, so it may have a somewhat lower local potency, but overall, all three (MIT·HCl, MIT and CMIT/MIT) are clearly corrosive, and MIT and CMIT/MIT have acute tox classifications for all three exposure routes. The available data do not point to large discrepancies among these three substances concerning (skin) corrosivity or oral acute toxicity. RAC agrees with the DS that read-across from MIT to MIT·HCl is less robust for acute toxicity than for the endpoints where corrosivity does not play a role. However, RAC is of the opinion that it would be difficult to disregard the MIT data leading to classification, even if there are uncertainties related to the HCl component potentially increasing the acute toxicity of MIT·HCl. Therefore, based on the available data, RAC considers the read-across from MIT to MIT·HCl acceptable. Especially considering that due to the corrosive nature of MIT·HCl, its testing for skin and inhalation acute toxicity has been waived in line with the BPR data requirements, so no further data on this exposure route and endpoint is foreseen.

The ATE is based on the lower MIT LD_{50} value of 242 mg/kg bw, corrected for molecular weight from MIT to MIT·HCl as 320 mg/kg bw.

In conclusion, based on the considerations described above, RAC is of the opinion that classification as **Acute Tox. 3, H311** (Toxic in contact with skin) is warranted, with an **ATE = 320 mg/kg bw**, based on read-across and a molecular weight corrected value from MIT.

Inhalation acute toxicity

Applying the same reasoning as for acute dermal toxicity, RAC considers that the read-across from MIT to MIT·HCl is acceptable also for the acute inhalation route.

The MIT LC50-values from the three acute inhalation toxicity studies were 0.11 mg/L, 0.134 mg/L and 0.19 mg/L. As concluded also in the RAC opinion for MIT from 2016 (CLH-O-0000001412-86-105/F), they all fall within the range given for dusts and mists in the classification criteria for category 2: $0.05 < \text{ATE} \leq 0.5$ mg/L. As there is no basis to disregard any of these values, RAC is of the opinion that the ATE should be based on the lowest value, 0.11 mg/L, corrected for molecular weight from MIT to MIT·HCl as 0.15 mg/L.

In line with the RAC opinion on MIT and the MIT·HCl data on skin corrosion, RAC considers that the most likely explanation for the observed inhalation toxicity is the corrosive nature of MIT·HCl (and MIT). Based on this, EUH071 ("Corrosive to the respiratory tract") should also be applied for MIT·HCl.

In conclusion, RAC is of the opinion that classification as **Acute Tox. 2, H330** (Fatal if inhaled) is warranted, with an **ATE = 0.15 mg/L**, based on read across and a molecular weight corrected value from MIT. In addition, RAC is of the opinion that the additional labelling phrase **EUH071 (Corrosive to the respiratory tract)** is warranted.

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

Summary of the Dossier Submitter's proposal

For STOT SE, the available data were the same as for acute toxicity (described under acute toxicity): One acute oral toxicity study performed with MIT·HCl, two acute dermal studies performed with MIT and three acute inhalation studies performed with MIT. No additional studies relevant to STOT SE classification were available.

The DS concluded that classification MIT·HCl with STOT SE 1 or 2 is not warranted (also MIT does not have a STOT SE classification). They noted that the acute toxicity studies available for MIT and MIT·HCl demonstrate acute toxicity, with the effects being attributable to local (site of contact) toxicity reflecting the corrosivity of the test material. Therefore, classification for STOT SE in Categories 1 and 2 is not relevant, as these effects are covered by the classification of the test material for acute toxicity and corrosivity.

Concerning STOT SE 3, the DS also concluded that no classification is warranted. They noted that the pulmonary effects (clinical signs and histopathological findings) seen in the acute inhalation studies can be attributed to the corrosivity of the substance, and that the corrosivity is covered by the skin corrosivity classification. They furthermore noted that MIT·HCl is thus also likely to be a respiratory irritant and should be labelled as EUH071, Corrosive to the respiratory tract.

The DS concluded that there are no data from acute toxicity studies that indicate a narcotic effect for MIT·HCl or MIT and that the clinical signs reported in acute toxicity studies are consistent with a local irritant effect at the site of contact (and secondary effects) and do not indicate any systemic effect on the nervous system.

Comments received during consultation

One MSCA commented the proposal, supporting no classification for STOT SE 1 and 2. They furthermore supported additional labelling with EUH071 (instead of STOT SE 3 (RTI) classification). However, regarding possible narcotic effects, they requested a more detailed discussion of the clinical signs reported in several studies that were considered by the DS as secondary to local toxicity (e.g. ataxia, hunched or prone posture, ptosis, passiveness, lethargy, prostration, hypoactivity, incoordination, squatting, abnormal gait).

In their reply (please see the RCOM), the DS noted that the clinical signs such as ataxia, hunched or prone posture, ptosis, passiveness/hypoactivity/lethargy, prostration, incoordination, squatting, abnormal gait and salivation are behavioural signs also commonly attributed to severe pain and an inflammatory response at the point of contact. MIT·HCl is a corrosive substance and the clinical signs reported in the acute toxicity studies are consistent with a local irritant effect at the site of contact (and secondary effects) that cause pain and do not indicate any systemic effect on the nervous system. Macroscopic changes observed at (usually) high doses confirmed irritant/corrosive effects on tissues at the point of contact.

Assessment and comparison with the classification criteria

The data available for STOT SE are the same that were summarised for acute toxicity. One oral acute toxicity study performed with MIT·HCl was available, the rest of the studies were performed with MIT or CMIT/MIT. No further data, for example from humans, was available for MIT·HCl (or MIT).

RAC considers the assessment from the RAC opinion for MIT from 2016 to be valid, concluding that: "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." Furthermore, in line with the assessment for MIT, although the data suggest that both MIT and MIT·HCl are respiratory irritants, the effects are accounted for by the classification for acute inhalation toxicity (Acute Tox. 2) and the application of the EUH071 phrase.

With regards to STOT SE 3 and narcotic effects, RAC concluded in 2016: "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." RAC still agrees with this assessment.

However, as mentioned above and also pointed out by the commenting MSCA in public consultation, also studies conducted in experimental animals can be considered for STOT SE 3. In several acute toxicity studies performed with MIT and CMIT/MIT, clinical signs (summarised under acute toxicity above) were observed that could, especially on their own, point to narcotic effects. However, RAC agrees with the DS that in this case these effects appear more likely to be secondary to the corrosive nature of the substance than due to narcotic effects following neurotoxicity. The effects, such as ataxia and incoordination, occurred in conjunction with many other clinical signs (indicating e.g. corrosion and presumably pain), rapidly after the start of exposure and at dose levels where also mortality was seen. Therefore, RAC is of the opinion that there is no reason to deviate from the conclusions made in the 2016 MIT opinion.

RAC also considers that there are no other data that would be sufficient to justify a STOT SE classification.

In conclusion, RAC agrees with the DS that **classification for STOT SE is not warranted.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

There was one GLP compliant *in vitro* test with MIT·HCl available (three-dimensional human skin model EPIDERM (OECD 431). Based on the result, the DS proposed to classify MIT·HCl for skin corrosion/irritation in Category 1A; H314: 'Causes severe skin burns and eye damage'. They noted that in a proportion of the cases, this *in vitro* test may result in over-classification, and considered Category 1A (H314) to cover the worst-case scenario.

Comments received during consultation

One MSCA agreed that based on the available data, Skin Corr. 1A could be assigned. However, they noted that considering the classifications for MIT and CMIT/MIT (Skin Corr. 1B and Skin Corr. 1C for C ≥ 0.6 %, respectively), it could be argued that a classification of MIT·HCl as Skin Corr. 1 without sub-categorisation may be more appropriate. They also noted that HCl (as hydrochloric acid … %) is classified as Skin Corr. 1B, with a specific concentration limit (SCL) (H314: C ≥ 25 %), which is higher than the generic concentration limit (GCL) (H314: C ≥ 5 %) allocated to MIT. In their response, the DS agreed that based on the argumentation presented in the comment, a classification as Skin Corr. 1 without sub-categorisation may be more appropriate.

Assessment and comparison with the classification criteria

The *in vitro* reconstructed human epidermis (RHE) skin model EpiDerm OECD 431 (2016) was used to test MIT·HCl for skin corrosion/irritation. In the test, 25 mg of MIT·HCl (purity 98.7 %) was applied to the skin model, no vehicle was used. Purified water was used as a negative control and 8N KOH as a positive control. The exposure times were 3 and 60 minutes followed by a 42-hour post-incubation period and immediate determination of cytotoxic effects via the MTT reduction assay. In the test, the negative and positive controls performed adequately. After the 3 min treatment, MIT·HCl produced a mean relative tissue viability of 22 %. After 60 mins, the mean relative tissue viability was 5.6 %.

According to the OECD test guideline 431, in step 1, a substance tested by the EpiDerm model is considered corrosive if viability is < 50 % after 3 min exposure (or ≥ 50 % after 3 min exposure AND < 15 % after 60 min exposure), as it was for MIT·HCl. Furthermore, in step 2 (for substances identified as corrosive in step 1), if the viability is < 25 % after 3 min exposure, the substance can be assigned an optional category 1A. It is noted in the guideline that according to the data generated in view of assessing the usefulness of the RHE test methods for supporting sub-categorisation, around 29 % of the category 1A results of the EpiDerm™ SCT may actually constitute category 1B or category 1C substances. If in step 2 viability after 3 min exposure is ≥ 25 %, a combination of optional categories 1B and-1C is suggested. Therefore, the result of the *in vitro* test indicated MIT·HCl as corrosive and predicted the category 1A (as optional). But it should be noted that the viability result of 22 % was bordering the limit of 25 %.

According to the guidance on the Application of the CLP Criteria (2017, p. 273), positive *in vitro* results on corrosivity do not generally require further testing and can be used for classification. It is mentioned that the RHE models included in the OECD TG 431 support the sub-categorisation into Category 1A, however they cannot discriminate between Categories 1B and 1C. Overall, it is stated that the RHE assays have been validated for the classification of skin corrosion and that the results of this validation are well founded.

Earlier, MIT was classified as Skin Corr. 1B based on weight of evidence. The data included two *in vivo* studies in rabbits. In addition, one *in vitro* study was available (human skin epidermal construct study EPIDERM, EPI-200 in accordance with OECD TG 431), which RAC considered to support the classification. Also a case report of a workplace accident with MIT was taken into account and considered to provide limited information. According to the RAC opinion CLH-O-0000001412-86-105/F, 2016 concerning the EPIDERM-study: "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."

Based on the EpiDerm studies performed for MIT·HCl and MIT, MIT·HCl would appear to have been more corrosive. However, comparison of these two studies is difficult, as MIT was tested dissolved in water, while MIT·HCl appears to have been tested in powder form.

CMIT/MIT has a classification as Skin Corr. 1C, H314, C ≥ 0.6 %, this was based solely on several *in vivo* tests (tested at different concentration levels in water).

HCl is classified as Skin Corr. 1B; H314: C ≥ 25 % and Skin Irrit. 2; H315: 10 % ≤ C < 25 %. Thus, the influence of the HCl in MIT·HCl dissolved in water may be limited.

RAC considers that the available *in vitro* study for MIT·HCl clearly demonstrates that it is a skin corrosive substance. The study also indicates that MIT·HCl could be sub-categorised into Category 1A. However, the data are less robust for sub-categorisation. Roughly 1/3 of substances for which the EpiDerm model indicates category 1A in reality fall into categories 1B/1C. In addition, the result for MIT·HCl was bordering the cut-off limit (cell viability 22 % vs. cut-off limit

for Cat. 1A < 25 %). Furthermore, only the solid state (powder) of the test substance was used in the study, aqueous solution was not tested at all. According to the DS, the pure/non aqueous MIT·HCl is only present in solid state immediately after manufacturing.

Overall, RAC is of the opinion that classification of MIT·HCl as **Skin Corr. 1, H314 is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

No data were available for serious eye damage/eye irritation. The DS noted that the registrant had submitted a waiver based on the available information indicating that the criteria are met for classification as Eye Dam. 1. The DS concluded that MIT·HCl is confirmed to cause skin corrosivity and proposed to classify as Eye Dam. 1; H318: 'Causes serious eye damage'.

Comments received during consultation

One MSCA agreed with the classification proposal but noted that labelling for serious eye damage in addition to skin corrosion is not necessary.

Assessment and comparison with the classification criteria

According to the CLP criteria, skin corrosive substances (Cat. 1/1A/1B/1C) shall be considered as also leading to serious damage to the eyes (Cat. 1). The corresponding hazard statement (H318: Causes serious eye damage) can be omitted on the label to avoid redundancy, as serious damage to the eyes is reflected in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage) (CLP regulation, Table 3.3.5 (1)).

As the data on skin corrosion/irritation is considered sufficient to classify MIT·HCl as corrosive to the skin, RAC agrees that also **classification as Eye Dam. 1 is warranted**. The corresponding hazard statement (H318: Causes serious eye damage) can be omitted.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS didn't propose a classification for respiratory sensitisation due to lack of relevant data in animals and humans.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Due to lack of relevant data this endpoint was not assessed, and RAC agrees with **no classification**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed to classify MIT·HCl as Skin Sens. 1A (H317: May produce an allergic skin reaction) based on read-across from MIT. They also proposed a specific concentration limit ≥ 15 ppm in line with that of MIT. No studies performed with MIT·HCl were available. The read-across was based on the following studies available for MIT:

- One LLNA study (OECD TG 429) in mice with 10.37 % MIT in water at 0, 1 500, 4 500, 7 600, 13 500, 15 700, 18 000 ppm.
- Seven studies and case reports in humans, which were considered as supportive.
- One *in silico* OECD (Q)Sar toolbox analysis that was considered as supportive.

In addition, the RAC opinion for MIT, adopted in March 2016, and the studies included there (and in the MIT CLH proposal) were summarised.

The read-across was justified by MIT·HCl being water soluble, meaning it will dissociate in physiological conditions encountered in the skin to form MIT and HCl. The MIT component derived from MIT·HCl is chemically indistinguishable from MIT and will therefore have the same effects regarding skin sensitisation.

Comments received during consultation

One MSCA supported the proposal. They also mentioned that four studies in guinea pigs (2 Magnusson-Kligman-tests, one Buehler-test, one open epicutaneous test) and one LLNA of the CLH report on MIT are missing. The DS replied that while these studies were not included in the table summarising the animal studies on skin sensitisation, they were mentioned in the text in Section A3.5.2 Comparison with the CLP criteria (reference to the RAC opinion for MIT).

One company-downstream user did not agree with the proposed SCL of 15 ppm, and instead proposed 100 ppm, with a corresponding EUH208 labelling limit of 10 ppm (0.001 %). They referred to reports showing the decrease in MIT sensitisation cases in Europe within the past ~10 years (e.g. Uter *et al*., 2020; The epidemic of methylisothiazolinone contact allergy in Europe: follow-up on changing exposures), and some reports describing the concentrations of MIT in various products (Garcia-Hidalgo *et al.,* 2017; Marrero-Aleman *et al.,* 2019) which they considered to provide support for the higher SCL of 100 ppm.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the read-across from MIT to MIT·HCl is suitable. It is not considered plausible that the HCl component of MIT·HCl would have an impact on this endpoint. There were no studies available on MIT·HCl itself.

The MIT data regarding skin sensitisation have been assessed extensively in the RAC opinion for MIT adopted on March 2016 (CLH-O-0000001412-86-105/F). RAC considers the assessment and comparison with the classification criteria performed then to still be valid. In conclusion, all available *in vivo* studies provide results that match the criteria for skin sensitisation classification in category 1A. The available human data provides further support to the results of the *in vivo* tests. No additional data were presented specifically for MIT·HCl.

Therefore, RAC agrees with the DS that **classification of MIT·HCl as Skin Sens. 1A, H317 is warranted**.

MIT has an SCL of 15 ppm, which is in line with the SCCS recommendation (2015) to limit the levels of MIT in rinse-off cosmetics to 15 ppm. This level is largely based on human data showing

cases of sensitization caused by products with MIT levels below 100 ppm. After introduction of this limit, the sensitization cases to MIT have decreased significantly in Europe as described e.g. in the paper by Uter *et al.* (2020). RAC does not consider that there are any new data which would support the higher SCL for MIT. The references cited in the consultation (Garcia-Hidalgo *et al.*, 2017; Marrero-Aleman *et al.,* 2019) on the concentrations of MIT in various products show that at time of the studies (2015-2017) variable levels of MIT were observed in different cleaning products, detergents and cosmetics, being in many cases < 50 ppm. In the study by Garcia-Hidalgo *et al*. (2017), half of the products tested were collected from sensitized patients. Thus, RAC does not consider these studies to provide evidence for the application of a higher SCL, as proposed by one commenter in the consultation, but to rather support the earlier SCL of 15 ppm for MIT.

In line with the RAC opinion on MIT (2016), 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.

In line with the SCL of MIT, RAC agrees with the DS that also for MIT·HCl, an **SCL ≥ 0.0015 % (15 ppm)** is warranted. 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 proposed to not classify MIT·HCl for STOT-RE. No studies were available for MIT·HCl itself; the proposal was based on read across from MIT and CMIT/MIT.

For oral exposure, four sub-chronic studies were available for MIT:

- Dietary exposure for 3 months daily in the Beagle dog (OECD TG 409, GLP), 53.2 % MIT (Kordek™ 573T) at dose levels of 0, 100/130, 400, 1 500 ppm (Anonymous 20, 2004)
- Drinking water exposure for 3 months in the Sprague-Dawley rat (OECD TG 408, GLP), 97.8 % MIT (RH-573 Technical) at 0, 75, 250, 1 000 ppm (Anonymous 21, 2000)
- Gavage exposure for 28 days in the Wistar rat (OECD 407, no mention of GLP), 49 % MIT (Acticide M50) at 0, 10, 28.6, 71.2 mg/kg bw/d (Anonymous 2, 2002)
- Gavage exposure for 90 days in the Wistar rat (OECD 407, no mention of GLP), 50.5 % purity MIT (Acticide M50) at 0, 7.52, 15.05, 30.09 mg/kg bw/d (Anonymous 3, 2002)

For dermal exposure, one sub-chronic study was available for CMIT/MIT:

- Dermal exposure for 3 months (5 days/week) in the New Zealand White rabbit (OECD 411, no mention of GLP), 14.6 % CMIT/MIT (equiv. 3.65 % MIT and 10.95 % CMIT; Kathon™ 886 MW) at 0, 100, 200, 400 ppm (Anonymous 24, 1982)

For inhalation exposure, one sub-chronic inhalation study was available for CMIT/MIT:

- Nose-only inhalation exposure for 3 months (6 h/d, 5d/week) in the Sprague-Dawley rat (OECD 413, GLP), 14.6 % CMIT/MIT (Kathon™ 886 MMP Process) at 0, 0.34, 1.15, 2.64 mg/m^3 (Anonymous 22, 1984)

In addition, two long-term studies were available for CMIT/MIT:

- Oral drinking water exposure study for 24 months in the Sprague-Dawley rat (OECD 453, CLP), 14.2 % CMIT/MIT (Kathon™ 886) at 0, 30, 100, 300 ppm (Anonymous 29, 1994)

- One dermal 30 months (3 days/week) study in the CD-1 mouse (OECD 452, GLP), 1.5 % CMIT/MIT (Kathon™ CG) at 0, 400 ppm

Overall, the DS concluded that the effects observed in the studies were either restricted to reduced body weights (oral exposure) or local effects (rhinitis in the inhalation and skin irritation in the dermal studies), with no functional changes observed. The effects observed on body weight gain and food consumption were considered by the DS as secondary to the local toxicity.

Comments received during consultation

One MSCA commented and agreed with the proposal to not classify for STOT RE.

Assessment and comparison with the classification criteria

The first four studies on MIT were included also in the MIT classification proposal and were assessed in the RAC opinion for MIT adopted on March 2016 (CLH-O-0000001412-86-105/F). RAC considers the assessment performed then to still be valid. In conclusion, there were no findings sufficiently serious to justify classification for repeated toxicity.

Four more studies on CMIT/MIT were included (dermal and inhalation repeated dose studies):

In Anonymous 24, 1982 (3-month dermal exposure study in the rabbit), 25-40 % of the rabbits died after 6-8 weeks of treatment across the low, mid- and high-dose groups. No deaths occurred in the controls. The DS attributed the deaths to pleuritis and alveolar edema, but noted that it cannot be ruled out that this effect was treatment related. A NOAEL was not determined due to evidence of concentration-related increase in local effects at all tested concentrations (slight to severe erythema, slight oedema). However, the DS noted that there was no evidence of systemic toxicity in this study. According to the DS, necropsy revealed minimal to slight sub-epidermal cell infiltrate in 8/12 rabbits at the highest dose level (400 ppm).

RAC considers that the aetiology of the observed effects in the lungs cannot be assessed based on the data available to RAC. However, similar systemic effects targeting the lungs were not seen in any of the other studies available. RAC considers that these effects cannot be viewed to fulfil the classification criteria for STOT RE.

In Anonymous 22, 1984 (3-month rat inhalation study), the DS noted that the high-dose group (2.64 mg a.i./m^3) exhibited symptoms consistent with those produced by a sensory irritant (chromorhinorrhea, rhinorrhea, eye squint, bradypnea, dyspnea). Also decreased body weight gains were apparent. Clinical chemistry revealed decreased serum protein in females at 2.64 mg/m^3. Decreased male spleen weights were seen at 2.64 mg/m^3. Histopathological evaluation showed slight to moderate incidences of eosinophilic droplets in the anterior respiratory mucosa of the nasal turbinates, and slight rhinitis in the lining of the anterior portion of the nasal cavity were observed in the 2.64 mg/m^3 treated animals. All the histopathologic changes were noted by the DS as very minor, potentially reversible, and generally reflective of minimal tissue responses to a very mild, low-grade respiratory irritant.

In Anonymous 29, 1994 (combined chronic rat toxicity/carcinogenicity study), reduced food consumption was seen in male rats exposed to 300 ppm. Also, a treatment-related and concentration-dependent decrease in water consumption was seen in both sexes in all treated groups throughout the study. The DS concluded that this appeared to be due to the unpalatability of the test material. Treatment-related morphologic changes were limited to the stomach and occurred in both sexes at 100 and 300 ppm. The primary effect noted was gastric irritation which was reflected by thickening of the forestomach mucosa due to hyperplasia and hyperkeratosis of the squamous mucosa. Focal necrosis of the superficial glandular mucosa and oedema and inflammatory cell infiltration in the forestomach submucosa were seen in the 300 ppm males.

In Anonymous 30, 1983 (30-month mouse dermal carcinogenesis study), according to the DS, effects were limited to brown staining, eschar, flaking and/or desiccation of the application site. Histopathology of the application site skin revealed focal or multifocal epidermal necrosis, hyperplasia, hyperkeratosis, eschar, dermal inflammation, and increased dermal collagen.

RAC agrees with the DS that also in these studies on MIT and CMIT/MIT, there were no findings sufficiently serious to justify classification for repeated toxicity. There were no studies available on MIT·HCl itself. Therefore, **no classification for STOT RE** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter evaluation is mainly based on read-across to MIT. According to the Dossier Submitter, the read-across is justified since the target substance (MIT·HCl) will dissociate to form MIT (the source substance) and HCl in the aqueous environment. The HCl generated from the dissociation of MIT·HCl is indistinguishable from the HCl present in the culture media or stomach and further dissociates to the hydrogen and chloride ions, causing no concern relating to the genotoxicity of the HCl component.

Dossier Submitter reported one in vitro bacterial gene mutation study with MIT·HCl and altogether four bacterial and mammalian gene mutation *in vitro* assays with MIT. All remained negative. There was an increase in the incidence of chromosome aberrations in one Chinese hamster ovary (CHO) cell study after MIT exposure. However, this finding was only observed at concentrations that induced significant cytotoxicity suggesting an indirect effect associated with cytotoxicity. Negative results were observed in two *in vivo* bone marrow micronucleus studies in mice. MIT did not increase the unscheduled DNA synthesis in primary rat hepatocytes.

Overall, the DS concluded that MIT·HCl does not fulfil the CLP criteria for germ cell mutagenicity.

Comments received during consultation

A member state representative agreed with the DS proposal and the read-across to MIT. The negative results of two in vivo micronucleus tests with MIT provide clear support for the no classification for genotoxicity even though based on toxicokinetic data MIT can reach target organs (also germ cells). In addition, negative findings in *in vivo* studies with MIT are further supported by negative *in vitro* results with the target substance MIT·HCl.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter's read-across approach to MIT. One *in vitro* mutagenicity study with MIT·HCl and six studies with MIT were described in the CLH Report. Negative results were reported for MIT·HCl in one and for MIT in two bacterial mutagenicity studies. Negative results were also reported for MIT in two gene mutation studies (HPRT) in CHO cells and in two chromosome aberration studies performed with CHO cells and human lymphocyte cultures.

No *in vivo* data are available for MIT·HCl. Negative results were observed in three *in vivo* studies available on MIT. In a micronucleus assay, the frequency of micronucleated polychromatic erythrocytes in the bone marrow of CD-1 mice was not increased by MIT. In an another study, a negative result was found using an alternative strain of mice (CRL:NM RI BR). The results of a rat liver unscheduled DNA synthesis (UDS) assay were negative. There was an additional UDS study reported on CMIT/MIT mixture which was also negative.

RAC agrees with the DS that **no classification of MIT·HCl for germ cell mutagenicity is warranted** based on read-across from the *in vitro* and *in vivo* mutagenicity studies performed with MIT.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS reported two combined chronic toxicity/carcinogenicity studies performed with CMIT/MIT mixture in the CLH report. One study was performed with orally exposed rats and other one with dermally exposed mice. Both studies remained negative. Like in the case of mutagenicity, the Dossier Submitter considered read-across justified since the target substance (MIT·HCl) will dissociate to form MIT (the source substance) and HCl in the aqueous environment. The HCl generated from the dissociation of MIT-HCl is indistinguishable from the HCl present in the stomach and is not considered to cause concern for carcinogenicity. In addition, the DS stated that CMIT is structurally similar to MIT and has a comparable toxicological profile and read-across to the CMIT/MIT mixture is additionally justified on this basis.

The DS proposed no classification on carcinogenicity for MIT·HCl as there is insufficient evidence for a carcinogenic effect in rats and mice (exposed with CMIT/MIT), and there are no other concerns about the potential carcinogenicity.

Comments received during consultation

A member state representative agreed with the DS proposal based on one oral and one dermal carcinogenicity study with CMIT/MIT mixture.

Assessment and comparison with the classification criteria

There are no studies on the chronic toxicity and carcinogenicity potential of MIT·HCl / MIT. Clear negative results from mutagenicity studies mainly on MIT do not raise concern for carcinogenicity. No indications from the repeated dose studies suggesting that MIT may potentially be carcinogenic by a non-genotoxic mechanism are available. Finally, two combined chronic toxicity/carcinogenicity studies (rat oral, mouse dermal) with CMIT/MIT mixture produced no evidence of carcinogenicity. RAC agrees with the DS's proposal that **no classification of MIT·HCl for carcinogenicity is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed read-across to MIT data for reproductive and developmental toxicity endpoints since there are no studies performed with MIT·HCl. The DS justified read-across on the basis that MIT·HCl dissociates to MIT following oral dosing. The available data is the same as already described in MIT classification proposal and RAC opinion from 2016 and included one two generation reproductive toxicity study in rats and two developmental studies in rats and one in rabbits.

No effects on fertility and sexual function were observed in a two-generation reproductive toxicity study. However, reduced body weight gain and reduced food intake were observed in parent

animals and their offspring. The decreased body weight, food consumption and food efficiency were considered to be associated with decreased water consumption due to adverse taste or smell of the test substance. Reproductive performance, parturition and spermatogenic endpoints were unaffected by treatment. There were no systemic or neurological effects observed in F0 and F1 parental animals or in F1 and F2 pups at any dose. No test-related macroscopic or microscopic changes neither effect on mean organ weights of F0 or F1 were observed at any dose. Based on reduced body weight and food consumption the parental and offspring toxicity NOAEL was 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). The NOAEL for reproductive toxicity was 1 000 ppm (69/93 mg/kg bw/d) which was the highest dose tested.

In the developmental toxicity studies, there were no developmental effects observed in either species exposed with MIT. Based on reduced body weight gain and reduced food consumption, the NOAEL for maternal toxicity in the first rat study was 20 mg/kg bw/d and the NOAEL for developmental toxicity was 40 mg/kg bw/d, which was the highest dose tested. In the second rat study, a maternal NOAEL of 33.4 mg/kg bw/d was acquired due to significantly reduced body weight gain. The NOAEL for developmental toxicity was 33.4 mg/kg bw/d, since higher and maternally toxic doses (50 and 75 mg/kg bw/d) increased the incidence of incomplete ossification and dilated cerebral ventricles. In a study with rabbits, a NOAEL of 10 mg/kg bw/d was determined for maternal toxicity based on dark red areas in the stomach, decreased defecation, body weight loss and reduced food consumption. A NOAEL of 30 mg/kg bw/d was determined for developmental toxicity which was the highest dose tested.

The DS concluded that no classification for reproductive and developmental toxicity is warranted according to the CLP Regulation.

Comments received during consultation

A member state representative agreed with the DS proposal but provided a comment on effects observed on sperm parameters in a 90-d oral toxicity study in Wistar rats. These changes in sperm parameters were, however, within HCD range and were not observed in the two-generation reproductive study.

Assessment and comparison with the classification criteria

There are no available studies on the reproductive and developmental toxicity of MIT·HCl. RAC agrees with the justification to use read-across for the reproductive toxicity classification of MIT·HCl on the basis that MIT·HCl dissociates to MIT in aqueous environment and following oral dosing.

Sperm parameter changes were observed in a sub-chronic 90-day oral toxicity study in rats exposed to MIT at doses of 7.52, 15 or 30 mg/kg bw/d by gavage. In all treatment groups, a statistically significant increase in mean percent of morphologically abnormal sperm cells was observed (0.67±0.66 % (mean±SD), 2.2±1.29 %, 2.35±1.25 % and 2.80±1.09 %, respectively). It was reported that 4.05 % of sperm cells in the control 28-day recovery group and 4.95 % in the high dose recovery group were morphologically abnormal. Historical control data from 2-generation studies by the same laboratory gave a mean value of 5.3 % for abnormal sperm meaning that the levels were still within the historical control data range. In the high dose group, sperm motility was reduced and a dose-dependent reduction in the number of testicular sperm heads was observed. No histopathological changes, no reduction in epididymal sperm count and no changes in testis weight were, however, observed.

In another 90-day oral toxicity study with rats (different strain), no comparable findings were reported (the highest dose was approx. 66/94 mg/kg in males/females).

No effects on fertility and sexual function were observed in a two-generation reproductive toxicity study in rats after MIT exposure. In this study, reproductive performance, parturition and spermatogenic endpoints were unaffected by MIT treatment (dose levels mg/kg bw/d: 0, 4-7 (males) and 6-13 (females); 15-19 (males) 22-26 (females); and 69-86 (males), 93-115 (females)). However, at the highest dose maternal and offspring toxicity was observed in the form of reduced body weight gain and reduced food intake.

No developmental toxicity was observed in two rat and one rabbit studies after MIT exposure.

In the first developmental study on rats the highest dose (60 mg/kg bw/d) exceeded the maximum tolerated dose and, therefore, the high dose was lowered to 40 mg/kg bw/d. The NOAEL for maternal toxicity was 20 mg/kg bw/day based on reduced body weight gain and reduced food consumption and the developmental NOAEL was 40 mg/kg bw/day. In the second developmental toxicity study in rats at higher doses, statistically significant and dose-dependent reduction in mean maternal body weight gain (16 % at 50 mg/kg bw/d and 30 % at 75 mg/kg bw/d) and food consumption were observed. The maternal and developmental NOAEL was 33.4 mg/kg. At maternally toxic doses (50 and 75 mg/kg bw/d) increased incidence of dilated cerebral ventricles and incomplete ossification were observed. These anomalies have been discussed in RAC opinion for MIT (CLH-O-0000001412-86-105/F, 2016) which concluded that the observations of dilated cerebral ventricles in rats are rather artefacts caused by the sectioning 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.

In rabbits, decreased defecation, dark red areas in the stomach, body weight loss and reduced mean food consumption were observed at 30 mg/kg bw/d. The maternal NOAEL was 10 mg/kg bw/day and the developmental NOAEL 30 mg/kg bw/day.

Based on the data presented above, the reproductive and developmental toxicity potential of MIT were evaluated in RAC opinion for MIT (CLH-O-0000001412-86-105/F, 2016) with the conclusion of no classification. In line with this, RAC agrees with the DS that **no classification for reproductive toxicity is warranted for MIT·HCl**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS's proposed classification as Aquatic Acute 1 (H400) with an M-factor of 1, based on a 72 h E_rC_{50} of 0.289 mg/L for *Pseudokierchneriella subcapitata* and Aquatic Chronic 1 (H410) with an M-factor of 1, based a 72 h NOE_rC of 0.047 mg/L for *P. subcapitata* both using data for MIT·HCl. MIT·HCl was also considered to be not rapidly degradable and have a low potential for bioaccumulation.

Summary

The DS noted that MIT·HCl dissociates in contact with water and becomes (de-) protonated according to the pH of the solution. Under typical use conditions, in which the active substance is highly diluted (e.g. maximum in-use concentration: 0.999 % w/w), the protonated form of the active substance will not depend on whether MIT·HCl or MIT (free base) was used as biocidal product. Consequently, the read across has been undertaken using the ECHA Read-Across Assessment Framework (RAAF, 2017a) from MIT (free base) to MIT·HCl.

The DS provided selected data (in italics for studies performed on the active substance MIT taken from the MIT CAR for PT 13 (November 2014) but did not use this read-across for their classification proposal, basing the proposal on data for MIT·HCl.

Degradation

Hydrolysis

Available data based on the test method US EPA N161-1, showed that MIT·HCl is hydrolytically stable at pHs 5, 7, and 9. The DS concluded that MIT·HCl is hydrolytically stable.

Phototransformation in water

Results from a study carried out under US EPA N161-2 guideline showed that MIT was photolytically degraded at a moderate rate with a half-life of 11.1 days. Two major metabolites were produced: 3-methyl-4-thiazolin-2-one and *N*-methyl malonamic acid.

Biodegradation

There was one ready biodegradability test available on MIT·HCl (OECD TG 301B, GLP), using test concentrations of 10 mg carbon/L in sealed culture vessels inoculated with activated sewage-sludge. The results obtained showed 0 % biodegradation in 28 days for test substance and 0 % biodegradation for control, indicating that MIT·HCl exhibits an inhibitory effect on the sewage treatment microorganisms used in the test. The ready biodegradability of MIT·HCl at lower concentration (as proposed in Annex II of the OECD guideline 301) was not tested, accepting that test results reflect the worst-case assumption for exposure assessment. The DS concluded that MIT·HCl cannot be considered as readily biodegradable according to OECD criteria.

Conclusion

Based on the available information, the DS concluded that MIT·HCl shows no evidence of mineralisation at a level ≥ 70 % and was considered to be not rapidly degradable.

Bioaccumulation

The DS considered the value for log K_{ow} -0.44 based on EPI SuiteTM estimation and concluded that MIT·HCl exhibits negligible potential for bioaccumulation. The same is valid for MIT with log K_{ow} value of -0.32 and BCF_{fish} of 0.107 L/kg (PT 11 Assessment Report, 2017).

Aquatic toxicity

The DS considered that MIT·HCl will dissociate in contact with water and become (de-) protonated according to the pH of the solution. In highly diluted solutions (e.g. maximum in-use concentration: 0.999 % w/w) the protonated form of the active substance will be identical with MIT (free base) already used and assessed as a biocidal product. The DS presented a justification for rad-across from MIT, this is described below as part of RAC's assessment.

The DS presented the assessment of aquatic toxicity taking into account available data for MIT·HCl, on acute toxicity to *Daphnia magna* (Anonymous, 2017a) and growth inhibition to algae (Anonymous, 2017b), and MIT (MIT CAR for PT 13, November 2014).

Acute aquatic toxicity

***Table**: Summary table – acute/short-term aquatic toxicity*

Guideline/Test method, Reliability	**Species**	**Test material**	**Endpoint**	**Exposure**		**Result**	Reference
				Design	Duration [h]		
Fish							
No data available							
Invertebrates						**EC_{50}**	
OECD TG 202, EC C.2 RI = 1	Water flea *Daphnia magna*	MIT·HCl (> 99.9 %)	Immobilisation	Static	48	2.33 mg/L, nominal	*Anonymous* (2017a) (Roche)
Algae						**E_rC_{50}**	
OECD TG 201, US EPA 122-2, EEC C.3 RI = 2	*Pseudokirchneriella subcapitata*	MIT (97.8 %)	Growth	Static	120	0.102 mg/L, initial measured (24 h)	Anonymous (1997) (Rohm and Haas)
OECD TG 201, EC C.3 RI = 1	*Pseudokirchneriella subcapitata*	MIT·HCl (> 99.9 %)	Growth	Static	72	0.289 mg/L, mean measured (72 h)	Anonymous (2017b) (Roche)

No short term toxicity data for MIT·HCl or MIT with fish were available in the dossier. Data from an acute static toxicity study with *D. magna* conducted according to OECD TG 202, EC C.2 are available (Anonymous, 2017a). The study meets the validity criteria and the DS considers the study valid. The nominal concentrations tested were 100, 45.5, 20.7, 9.4, 4.3, 1.9 and 0.9 mg test MIT·HCl/L and a control. Analytical determination was performed in samples taken on 0 and 48 h and all reported results refer to nominal values since the concentrations of the test item were within ±20 % of the nominal concentrations during the test. The 48 h EC_{50} from the study is 2.33 mg MIT·HCl/L, based on nominal concentrations.

The DS presented two toxicity tests with the freshwater alga *P. subcapitata*, one conducted with MIT and one conducted MIT·HCl. Both met the validity criteria, with the DS considering both reliable.

The first test (Anonymous, 1997) showed that MIT is very toxic to freshwater algae. In this study, 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. In the beginning of the test MIT is rapidly taken up by the algae and inhibits enzymes by binding to the thiol-groups of the proteins. Due to this binding, cleaving of the isothiazolinone ring takes place degrading MIT. This explains why MIT concentration in the test decreased markedly in the beginning of the test with this decrease depending on algal cell density. This also changes the sensitivity of the test. In addition, concentration changes affected more strongly low test concentrations in comparison with high concentrations. The removal of test substance is rapid, and endpoint values based on geometric mean concentration does not take into account the biodegradation of MIT. For this reason, the 24 h E_rC_{50} based on initial measured concentrations was used as an endpoint in this study.

The second study (Anonymous, 2017b) conducted following GLP and used MIT·HCl as the test item on *P. subcapitata* was also available. The nominal concentrations tested were 1.00, 0.32, 0.10, 0.032 and 0.01 MIT·HCl/L. Analytical determination was performed in samples taken at 0 and 72 h. The samples taken on 0 h the measured concentrations varied from 95 % to 103 % of nominal, while a decline of the test substance concentration was observed thereafter, for in the samples taken at 72 h the measured concentrations varied from 14 % to 100 % of nominal.

Similarly, as was the case for MIT, at higher test concentrations, degradation of test substance was slower in comparison with lower test concentrations. The endpoints were based on the geometric mean measured concentrations of the test item: 1.00, 0.28, 0.047, 0.012 and 0.004 mg/L. The E_rC_{50} and the E_yC_{50} after 72 h were determined to be 0.289 mg/L and 0.112 mg/L MIT·HCl. It was noted that although both of the dose response curves for growth rates and yield were of good quality (monotonous) the EC_{10}, EC_{20} and EC_{50} values for growth rate were accompanied by 95 % confidence intervals (unbound low intervals and non-determined high intervals in all cases) that indicate some quality issues regarding their statistical robustness. The E_rC_{50} at 24, 48 and 72 h were determined to be 0.825, 0.249 and 0.290 mg/L, respectively, by the DS. These results illustrate that after 24 h a decrease in concentration is accompanied by a decrease in growth inhibition, indicating that recovery starts during the test.

The 72 h E_yC_{50} was calculated to be 0.112 mg/L and the 72 h E_rC_{50} value was calculated to be 0.289 mg/L. All reported results refer to geometric mean measured concentrations, since the test item concentrations were not within ±20 % of the nominal concentrations during the test. Analytical determination was performed in samples taken on 0 and 72 h. Decline of the test substance concentration was observed: nominal measured concentrations were from 95 % to 103 % of nominal, while in the samples taken at 72 h the measured concentrations varied from 14 % to 100 % of nominal. All validity criteria were met and the DS considered the study reliable.

Aquatic chronic toxicity

***Table**: summary chronic/long-term aquatic toxicity*

Guideline / Test method, Reliability	**Species**	**Test material**	**Endpoint**	**Exposure**		**Results**		Reference
				Design	Duration [d]	NOEC	EC_{10}	
Fish								
OECD TG 210, US EPA OPPTS 850.1400, US EPA 72-4, US EPA 797.1600 RI = 1	Rainbow trout *Oncorhynchus mykiss*	MIT (as a formulated product, 51.252 % in water)	Growth, wet weight	Flow-through	98	2.38 mg/L, mean measured	4.93 mg/L, mean measured	Anonymous (2005) (Rohm and Haas)
Invertebrates								
OECD TG 211, US EPA OPPTS 850.1300 RI = 1	Water flea *Daphnia magna*	MIT (as a formulated product, 51.252 % in water)	Dry weight	Flow-through	21	0.0442 mg/L, mean measured	0.0889 mg/L, mean measured	Anonymous (2004) (Rohm and Haas)
Algae								
OECD TG 201, US EPA 122-2, EEC C.3 R = 2	*Pseudokirchneriella subcapitata*	MIT (97.8 %)	Growth	**Static**	**120**	**-**	0.062 mg/L, initial measured (24 h)	Anonymous (1997) (Rohm and Haas)
OECD TG 201, EC C.3 RI = 1	*Pseudokirchneriella subcapitata*	MIT·HCl (> 99.9 %)	Growth	Static	72	0.047 mg/L, mean measured (72 h)	0.208 mg/L, mean measured (72 h)	Anonymous (2017b) (Roche)

A GLP early life stage chronic toxicity study with MIT on rainbow trout (*Oncorhynchus mykiss*) in line with OECD TG 210 was available (Anonymous, 2005). The study was conducted under flow-through conditions (for 98 days (62 days post-hatch)) and met the OECD TG 210. The nominal concentrations tested were 20, 10, 5, 2.5, 1.3, and 0.63 mg/L MIT and a control (4 replicate vessels per treatment and 15 animals/vessel) with measured concentrations: < LoD (Limit of

Detection) (control), 0.613, 1.20, 2.38, 4.93, 9.88, and 20.0 mg/L MIT. The $NOEC_{growth}$ was determined to be 2.38 mg/L MIT.

A GLP chronic toxicity study with MIT on *D. magna* following OECD TG 211 was available (Anonymous, 2004). The study was conducted under flow-through conditions (for 21 days) and met all the OECD TG 211 validity criteria. The nominal concentrations tested were 0.4, 0.2, 0.1, 0.05, 0.025, and 0.013 mg/L MIT and a control (4 replicate vessels per treatment and 10 animals (< 24 h old)/vessel) with measured concentrations: 0.0117, 0.0209, 0.0442, 0.0889, 0.183 and 0.359 mg/L (mean values from analysed samples taken on days 0, 7, 14 and 21). The lowest chronic value from this study is the 21-day $NOEC_{growth}$ of 0.0442 mg/L, for dry weight. However, it should be noted that growth is an optional test parameter according to OECD TG 211.

Chronic endpoints were available for the 2 algae tests described in detail above under acute toxicity.

The first test (Anonymous, 1997), the 24 h E_rC_{10} based on initial measured concentrations was used as an endpoint in this study.

For the second study (Anonymous, 2017b), it was noted that although both of the dose response curves for growth rates and yield were of good quality (monotonous) the EC_{10}, EC_{20} and EC_{50} values for growth rate were accompanied by 95 % confidence intervals (unbound low intervals and non-determined high intervals in all cases) that indicate some quality issues regarding their statistical robustness. The 72 h NOE_rC was determined to be 0.047 mg test item/L. All reported results refer to geometric mean measured concentrations, since the test item concentrations were not within ±20 % of the nominal concentrations during the test. Analytical determination was performed in samples taken on 0 and 72 h. Decline of the test substance concentration was observed: nominal measured concentrations were from 95 % to 103 % of nominal, while in the samples taken on 72 h the measured concentrations varied from 14 % to 100 % of nominal.

<u>DS classification conclusions</u>

- *Data selection*

The DS concluded that algae are the most sensitive trophic level based on the available data with MIT and MIT·HCl, with all derived E_rC_{50} values < 1 mg/L. The DS selected as the most reliable data Anonymous (2017b) as the test item is MIT·HCl and thus supersedes read-across data obtained with MIT (Anonymous, 1997). In addition, the DS noted that the endpoint from this study is a 72 h E_rC_{50} value for which the test item concentration is maintained well in the static system (98 % on 0 h and 78 % on 72 h of the nominal concentration of 0.32 mg/L). The DS also noted also that the 24 h E_rC_{50} endpoint defined for MIT is not the appropriate timescale and it is based on initial measured values due to substance degradation.

- *Acute toxicity*

The DS concluded that based on 72 h E_rC_{50} of 0.289 mg/L for *P. subcapitata*, MIT·HCl meets the CLP Regulation criteria for being classified as Aquatic acute 1 with an M-factor of 1.

- *Chronic toxicity*

The DS compared endpoints for NOEC for MIT·HCl and MIT taking into account different molar masses of both compounds for all trophic levels and concluded that algae are the most sensitive trophic group.

The DS concluded that based on 72 h NOE_rC of 0.047 mg/L for growth rate for *P. subcapitata,* and a lack of rapid degradation, MIT·HCl meets criteria (< 0.1 mg/L) for being classified as Aquatic chronic 1 with an M-factor of 1.

Comments received during consultation

One National Authority (NA), one company/manufacturer and one Member State (MS) commented on the proposed classification of MIT·HCl.

The NA noted assumptions already agreed by RAC for other isothiazolinones (MIT, OIT, MBIT, DCOIT, BIT), such as the use of 24- or 48-hour acute algal endpoints based on initial measured concentrations for hazard classification due to mean measured concentrations reflecting a significant period of time when the test substance is not available. Based on these prior conclusions, the NA asked the DS to provide acute and chronic endpoints for 24-, 48- and 72-h exposure periods based on initial measured and nominal concentrations for the study with MIT·HCl. In line with previous RAC assessments, the NA proposed the lower of 24- or 48-hour endpoints to be used as a base for acute classification. For the chronic classification, the NA proposed that the DS or RAC confirm which of these endpoints represent exponential growth and multiple generations and might be the most suitable for chronic classification. In addition, the NA noted that all relevant aquatic toxicity studies available in the CLH report for MIT should be included in the current CLH assessment. The lowest 24 h ErC_{50} of 0.0695 mg/L (initial measured concentrations) obtained for *Skeletonema costatum* as the most sensitive species should be taken into account that derives a higher acute M-factor. Additionally, all read-across data should be recalculated for the molar mass of MIT·HCl.

The company/manufacturer underlined that dissociated MIT·HCl and MIT are free base and conjugated acid and that the equilibrium between them depends only on pH. Consequently, all the data available for MIT should be used for the classification of MIT·HCl and fully consistent classification should be derived for both chemical forms. The company/manufacturer disagreed with DS to use Anonymous (2017) for the hazard classification of MIT·HCl and disagreed with the proposed classification as Aquatic Acute 1 with lower M-factor. The company/manufacturer is of the opinion that an algae study with *S. costatum* with an EC_{50} of 0.0695 mg/L (A7.4.1.3.b/01 from Rohm and Haas) should be taken into account for the classification of MIT·HCl and this would lead to a consistent harmonised classification of both substances as Aquatic Acute 1, M-Factor 10.

The MS noted that the most sensitive species and endpoint for *S. costatum* with an EC_{50} of 0.0695 mg/L (A7.4.1.3.b/01 from Rohm and Haas) has not been taken into account. The MS proposed all data for MIT to be used for classification and M-factor of 10 for the aquatic acute classification of MIT·HCl to be consistent with the classification of MIT with M-factor of 10.

In response to the received comments, the DS noted that read across approach to MIT is principally justified, is not used as data for MIT·HCl itself is preferred. The DS indicated that Anonymous (2017b) should be used for classification and takes precedence over read-across to MIT. The DS considered that the decline of the test substance concentration MIT·HCl is much slower compared to MIT and endpoints derived over 72 h (E_rC_{50} 0.290 mg/L) and 48 h (0.249 mg/L) are clearly lower compared to a 24 h endpoint (E_rC_{50} 0.825 mg/L). The DS concluded that this clearly indicates that there is a difference between environmental fate and intrinsic effects of MIT·HCl and this justifies not using read-across from MIT. The DS considered that exponential growth is ensured for 24, 48 h and only for 72/96 h does exponential growth drop off due the population growing too quickly. However, chronic classification could clearly be represented by 24 h and 48 h endpoints, as in the case for acute classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider MIT·HCl as 'not rapidly degradable', based on the 0 % degradation in a 28 day ready biodegradability test (OECD TG 301B) and no other data showing

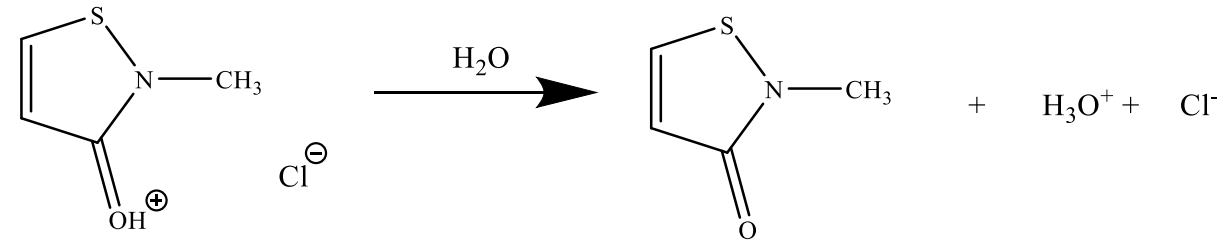
degradation ≥ 70 % after 28 days. MIT·HCl is not subject to hydrolysis in the classical sense but instead dissociates in aqueous solution, this is discussed below.

Bioaccumulation

RAC agrees with the DS that MIT·HCl has a low potential for bioaccumulation based on the calculated value of log K_{ow} of -0.44 (EPI SuiteTM estimation). Available data for MIT (log K_{ow} value of -0.32 and BCF_{fish} of 0.107 L/kg) also supports this conclusion.

Read-across from MIT

RAC has assessed the read-across argument as presented by the DS. RAC notes that MIT·HCl is a salt obtained after protonation of free base MIT with HCl. The substance MIT is an amide including a protonated oxygen (as $=OH^+$). Furthermore, in the presence of water, MIT·HCl will dissociate following the reaction:



MIT·HCl MIT

The equilibrium between free base MIT and the conjugated acid $MITH^+$ in aqueous solution depends only on pH and for pH values > 8 (e.g., in an algae test) will always tend to MIT. Consequently, all aquatic toxicity results obtained for MIT should be taken into account for the classification of MIT·HCl and RAC concludes to read-across from MIT (base) to the new active substance MIT·HCl. The endpoint values used for the classification of MIT are considered as valid for classification and should also be used for the classification of MIT·HCl.

Aquatic toxicity

Data and endpoint selection

RAC disagrees with the DS that the study with MIT·HCl (Anonymous, 2017b) takes precedence over read-across from MIT. Both substances are in pH mediated equilibrium in aqueous solution. At pH 8-9 (algal test), free base MIT is the relevant moiety where either test substance is used. RAC also disagrees with the DS that the decline of MIT·HCl concentration is much slower (Anonymous, 2017b) in comparison to MIT. The explanation is that in this case initial cell number is 2× lower (5 000 rather than 10 000) in Anonymous (2017b), using MIT·HCl. Decline of concentrations of the substance depends on cell number. There is no data for detection limits, but it might be assumed that the detection limit in Anonymous (2017b) can ensure measurements at lower levels and calculations for longer time periods.

RAC agrees with the DS that algae are the most sensitive organism for MIT, and this is also the case for MIT·HCl. An identical mode of action is to be expected for both MIT·HCl and MIT. Both compounds are able to diffuse across the cell membrane and in the intracellular media, the electron-deficient sulphur of the N–S bond reacts with the nucleophilic groups of the cellular components, such as the thiols from cysteines of protein active sites, blocking their enzymatic activity and ultimately causing cellular death. A consequence of this reaction is cleaving of the isothiazolinone ring and substance degradation. Indeed, this mechanism is described for bacteria and fungi but is also the case for algae. In conclusion, the inhibitory mechanism behind algae growth inhibition will also result in a degradation of MIT/MIT·HCl by algae. This explains the remarkable decrease of test substance concentration in the test media at the beginning of the

test, with the degree of decrease depending on algal cell density (the decrease is more pronounced at low test concentrations).

Consequently, the effect of MIT on the growth pattern is mainly related to the effects in the early phase of the exposure, which caused a lag phase in the cultures with the highest test concentrations. The 72 or 96 h NOEC based on nominal concentrations cannot be used as an endpoint for environmental risk assessment, as the removal of MIT from the test system is rapid. Using a NOEC based on geometric mean concentration does not take account of the interaction between algal density and biodegradation of MIT. It does not fully compensate for the fact that recovery of algal growth is taking place during the course of the studies. Using time points below 72 h is not a standard approach, as the general recommendations of the OECD TG 201 are to use the 72 h interval with a possibility to reduce the duration to 48 h. MIT is a fast-acting biocide and toxicity is stoichiometric and closely associated with degradation. For MIT, the 24 h E_rC_{50} based on initial measured concentrations is proposed as the appropriate endpoint for classification of acute hazard. For classification of chronic hazard the 24 h NOE_rC or E_rC_{10} based on initial measured concentrations should be used as endpoint from these studies. In addition, it should also be considered that both substances are stable in acidic media and highly unstable in alkaline solution: the instability increasing with increasing of pH. This means that comparison of endpoints for the same algal species is possible only for the same cell number and for the same pH of test media.

***Table**: Summary of results for aquatic toxicity for algae with both MIT and MIT·HCl. All tests use a static design with results for growth inhibition.*

Guideline/Test method	Species	Test material	Results		Reference
			NOEC/EC_{10}	EC_{50}	
OECD TG 201 US EPA FIFRA 122-2 EEC C.3	*Pseudokirchneriella subcapitata* Initially 10 000 cell/mL Test duration 120 h	MIT > 97.8 % RH-573 technical	24 h E_rC_{10} 0.062 mg/L, initial measured	24 h E_rC_{50} 0.102 mg/L	Anonymous (2004) (A7.4.1.3.b/01; Rohm and Haas)
US EPA FIFRA 123-2 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata* Initially 10 000 cell/mL Test duration 96 h	MIT	24 h E_rC_{10} 0.024 mg/L, initial measured	24 h E_rC_{50} 0.114 mg/L	(A7.4.1.3-01, Thor GmbH)
US EPA FIFRA 123-2	*Skeletonema costatum* Initially 10 000 cell/mL Test duration 120 h	MIT 51.25 %	24 h E_rC_{10} 0.044 mg/L, initial measured	24 h E_rC_{50} 0.0695 mg/L	Anonymous (2004) (A7.4.1.3.b/01; Rohm and Haas)
ISO 10253 OPPTS 850.5400	*Skeletonema costatum* Initial cell density 1 000 cell/mL Test duration 96 h	MIT	24 h E_rC_{10} 0.0727 mg/L, initial measured	24 h E_rC_{50} 0.1945 mg/L	Thor GmbH
OECD TG 201, EC C.3	*Pseudokirchneriella subcapitata* Initially 5 000 cell/mL Test duration 72 h	MIT·HCl > 99.9 %	72 h E_rC_{10} 0.208 mg/L, mean measured	72 h E_rC_{50} 0.289 mg/L, mean measured	Anonymous (2017b) (Roche)

The tests for MIT are described in detail in the CLH report, with additional tests using MIT mentioned during the consultation available in the previous RAC opinion for MIT (March, 2016).

Algae are the most sensitive species with four toxicity studies for MIT being available: two using *P. subcapitata* and two using *S. costatum*, with the remaining study for *P. subcapitata* being conducted with MIT·HCl.

All tests are valid and relevant for hazard classification.

In the first toxicity test using MIT *P. subcapitata* (Rohm and Haas), the concentration of test substance was not maintained at > 80 % of nominal concentrations during the test due to the previously described algal mediated degradation. The studied concentrations are 0.0503; 0.104; 0.202; 0.407, and 0.708. The initial cell density was 10 000 cell/mL. At the lowest test concentration, MIT was completely degraded within 72 h and not found above the limit of detection. Degradation is relatively slower at the highest test concentrations. The E_rC_{50} after 120 h of 0.220 mg/L from the study is twice the concentration with 50 % inhibition in the first 24 h of the test. Calculated values for the E_rC_{50} after 24, 48, and 72 h following independent statistical analysis are 0.103, 0.137 and 0.157 mg/L, respectively. This illustrates that inhibition decreases with time due to the decline in exposure to the test substance and recovery starts during the test. Variability of growth rate in the control is rather high for the first 24 h. The test does not fulfil the criterion that the mean coefficient of variation for section by section growth rates (day 0-1, 1-2, 2-3, for 72-hour tests) in the control cultures must not exceed 35 %. However, this validity criterion is in the updated OECD TG 201 (2006) and was not applicable at the time the study was performed (1997). It was however agreed that this study is acceptable. The 24 h E_rC_{50} of 0.102 mg/L based on initial measured concentrations is proposed as the appropriate endpoint for acute hazard classification. For classification of chronic hazard the 24 h E_rC_{10} based on initial measured concentrations (0.062 mg/L) should be used as the chronic endpoint.

In the second toxicity study of MIT with *P. subcapitata* (A7.4.1.3-01, Thor GmbH), the concentration of test substance was not maintained at > 80 % of nominal concentrations in the tests, due to algal mediated degradation. At the lower test concentrations, MIT was completely degraded within 96 h and not found above the limit of detection at the end of the test. Similar to the first test, degradation is relatively slower at the highest test concentrations. The exponential increase in cell density in the controls was not maintained after 72 h. Variability in the performance of controls becomes too high to detect a significant difference with the exposed test media. Evaluation of the study indicates that the effect on the growth pattern is mainly related to the effect in the early phase of the exposure due to the fact that concentrations of MIT are declining over time. E_rC_{50} values increased from 0.114 mg/L over the first 24 h to 0.118 mg/L after 48 h and 0.160 mg/L after 72 h. The 24 h E_rC_{10} of 0.024 mg/L is proposed as the value for the chronic classification.

The toxicity of MIT towards the marine alga *S. costatum* was tested (Rohm and Haas) (Anonymous, 2004). The test substance concentrations were measured at 0 and 120 h. Except for the media with the two highest initial test concentrations, all concentrations were below detection limit at 120 h. Due to the degradation of MIT and the lack of MIT measurements between 0 and 120 h, the 96 h NOE_rC of 0.0725 mg/L (based on initial measured concentrations) cannot be considered a reliable endpoint. The 24 h E_rC_{50} of 0.0695 based on initial measured concentrations is proposed as the appropriate endpoint for classification of acute hazard, this value was used to classify MIT by RAC in 2016. The 24 h E_rC_{10} is proposed as a supporting value for the chronic classification.

The second toxicity test using *S. costatum (*Thor GmbH*)* was performed for 96 h. In the study report authors noted that the organism forms chains containing several cells. The electronic particle counter counts particles and not individual cells, which is why the inoculum particle density was corrected for the mean chain length measured in the pre-culture. As the algal particle size varied during the test authors decided to base the calculation of the endpoints on the algal biovolume in the cultures. The initial cell density of 1 000 cells/mL is not comparable with

standard studies done with the freshwater alga *P. subcapitata* and previous test with *S. costatum.* Initial measured concentrations are 94-104 % of nominal. Based on the analytical results after 72 h concentration of MIT declined in all test systems, but most markedly in the test systems with the lower test concentrations However, analytical results after 96 h are contradictory as E_rC_{10}, $E_rC_{50,}$ and NOE_rC values increase with time. The NOEC derivation for the first 24 h has a high sensitivity due to a low variability of control performance and the fact that six control replicates were used. There is a clearer dose response for the first 24 h compared to 48 and 72 h. The results at the 0.357 mg/L nominal test level suggest inhibitory effects on algal growth up to 48 h following strong inhibition of growth during the first 24 h. This suggests a long-term effect but is most likely an artefact of the small size of the test system. The strong inhibition of growth during the first 24 h maintains a high ratio of MIT molecules compared to the number of algal cells. Such long-term effects would not be seen under more realistic conditions where the number of algal cells is not a limiting factor.

The final study using *P. subcapitata* (Anonymous, 2017b) (Roche) indicted only endpoint values for 72 h, giving an E_rC_{50} of 0.298 mg/L and E_rC_{10} of 0.208 mg/L using MIT·HCl. However, despite being reliable RAC does not agree to use this study for classification purposes as data for MIT should be used instead, using 24 h endpoints for comparison with the CLP criteria, due to the reasons explained above.

Comparison with CLP criteria

- *Acute aquatic hazards*

The lowest available $L(E)C_{50}$ value relevant for classification of MIT·HCl is the 24 h E_rC_{50} of 0.0695 mg/L obtained for the marine alga species *S. costatum* using MIT from Anonymous (2004), as mentioned in the consultation by all commenters. RAC agrees with commenters that as use of data from MIT is justified in this case, classification should be based on data from MIT (discussed above). Furthermore, as the toxic mode of action leads to the rapid breakdown of the test material, use of initial measured concentrations after 24 h is also justified. This approach has been followed by RAC for other isothiazolinones. Based on this value, MIT·HCl meets the criteria for classification as **Acute Aquatic 1**, **H400** (Very toxic to aquatic life) with an **M-factor of 10** due to the 24 h E_rC_{50} in the range $0.1 < L(E)C_{50} \geq 0.01$.

- *Aquatic chronic hazards*

The lowest $NOEC/EC_{10}$ is the 24 h E_rC_{10} of 0.024 mg/L obtained for the freshwater alga species *P. subcapitata* using MIT. RAC also notes that the 24 h E_rC_{10} of 0.044 mg/L from Anonymous (2004) would result in the same classification. The classification warranted for MIT·HCl will be **Aquatic Chronic Category 1**, **H410 (**Very toxic to aquatic organisms with long lasting effects) with an **M-factor of 1** taking into account that the substance is not rapidly degradable.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Stratospheric ozone depletion can be excluded due to the very short half-life in the air. MIT·HCl is predicted to have an atmospheric half-life of 14.35 h. Atmospheric half-lives reported in the MIT PT 11 Assessment Report (2017) are similar, i.e. 16.6 h (SAR method) and 14.3 h (AOPWIN™). Additionally, as the molecule does not contain olefinic carbon-carbon double or triple bonds, MIT·HCl is not expected to react with ozone.

The DS concluded that MIT·HCl does not fulfil the criteria for classification according to the criteria of Regulation (EC) 1272/2008. The half-life of MIT·HCl is relatively short and is therefore not considered to be involved in ozone depletion.

Comments received during consultation

No comments concerning hazards to the ozone layer were received.

Assessment and comparison with the classification criteria

RAC notes that MIT·HCl is not listed in Annex I of Annex II to Regulation (EC) No. 1005/2009 and that an Ozone Depleting Potential (ODP) is not reported for MIT·HCl.

RAC agrees with the DS that MIT·HCl does not contain moieties or phys-chem properties indicating a hazard to the ozone layer and concludes that data presented **do not warrant classification for the ozone layer**.

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 and additional information (if applicable).

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