Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

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

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

CLH-O-0000001412-86-105/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 March 2016

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name:

2-methylisothiazol-3(2H)-one

EC Number: 220-239-6

CAS Number: 2682-20-4

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-METHYLISOTHIAZOL-3(2H)-ONE (ISO)

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance 2-methylisothiazol-3(2*H*)-one

Table 1: Substance identity

Substance name:	2-methylisothiazol-3(2*H*)-one (MIT)
Common name, synonym:	MIT, MI, methylisothiazolinone, 2-methyl-4-isothiazoline-3-one, 2-methyl-2*H*-isothiazol-3-one
Commercial name :	Kordek™ 573T Industrial Biocide, RH-573, Kordek ™ 573F, ACTICIDE® M 20, ACTICIDE® M 20 S, ACTICIDE® M 50
EC number:	220-239-6
CAS number:	2682-20-4
Annex VI Index number:	currently not in Annex VI
Degree of purity:	> 95%
Impurities:	Confidential.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	currently not in Annex VI
Current proposal for consideration by RAC	**Classification:** Acute Tox. 3 (oral) , H301 Acute Tox. 3 (dermal), H311 Acute Tox. 2 (inhalation), H330 Skin corr. 1B, H314 Skin sens. 1A, H317 Aquatic Acute 1, H400 Aquatic Chronic 1 H410

	Specific concentration limits: Skin. Sens 1; H317: SCL ≥ 0.06 % **Acute M factor:** M=10 **Chronic M factor**: M=1 **<u>Labelling:</u>** GHS06, GHS05, GHS09 H301, H311, H330, H314, H317, H410, EUH071, Dgr
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	**<u>Classification:</u>** Acute Tox. 3 (oral) , H301 Acute Tox. 3 (dermal), H311 Acute Tox. 2 (inhalation), H330 Skin corr. 1B, H314 Skin sens. 1A, H317 Aquatic Acute 1, H400 Aquatic Chronic 1 H410 **Specific concentration limits:** Skin. Sens 1; H317: SCL ≥ 0.06 % Acute M factor: M=10 Chronic M factor: M=1 **<u>Labelling:</u>** GHS06 , GHS05, GHS09 H301, H311, H330, H314, H317, H 410, EUH071, Dgr

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 3 (oral) , H301	Not applicable	Not classified	
	Acute toxicity - dermal	Acute Tox. 3 (dermal), H311	Not applicable	Not classified	
	Acute toxicity - inhalation	Acute Tox. 2 (inhalation), H330	Not applicable	Not classified	
3.2.	Skin corrosion / irritation	Skin corr. 1B, H314	Not applicable	Not classified	

3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	
3.4.	Skin sensitisation	Skin sens. 1A, H317	H317 : SCL ≥ 0.06 %	Not classified	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1 H410	M=10 M=1	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictograms: GHS06, GHS05, GHS09

Signal word: Dgr

Hazard statements:

H301; Toxic if swallowed.
H311; Toxic in contact with skin.
H330; Fatal if inhaled.
H314; Causes severe skin burns and eye damage.
H317; May cause an allergic skin reaction.
H410; Very toxic to aquatic organisms with long lasting effects
EUH071; Corrosive to the respiratory tract.

Proposed notes assigned to an entry:

Specific concentration limits:
Skin. Sens 1; H317 : SCL ≥ 0.06 %
High skin sensitisation potential of MIT warrants specific concentration limits for skin sensitisation.
M factor

An **acute M factor of 10** will be applied, due to the 24 hours E_rC_{50} of 0.0695 mg/l from the *Skeletonema costatum* study. An **chronic M factor of 1** will be applied, due to the 24 hours E_rC_{10} of 0.024 mg a.i./l from the *Pseudokierchneriella subcapitata* study.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

A harmonised classification for 2-methylisothiazol-3(2H)-one in not availble and the substance is not listed in Annex VI of the Regulation (EC) No 1272/2008. 2-Methylisothiazol-3(2H)-one (MIT) is a biocidal active substance that has been evaluated in the context of the work programme for review of existing active substances provided for in Article 89 of the Regulation (EU) No 528/2012 with a view to the possible approval of this substance for use as a metalworking-fluid preservative (product-type 13).

2.2 Short summary of the scientific justification for the CLH proposal

This classification proposal is based mainly on the hazard assessment of the substance presented in the Document II A and the Document III A of the Competent Authority Report (CAR).

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

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Substance methylisothiazol-3(2H)-one is not listed in Annex VI of the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Substance methylisothiazol-3(2H)-one is not listed in Annex VI of the CLP Regulation.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification and labelling applied by most companies is following:

Classification:

Acute Tox. 3 (oral),	H301
Acute Tox. 3 (dermal),	H311
Skin corr. 1B,	H314

STOT SE 3,	H335
Eye Dam. 1,	H318
Skin sens. 1,	H317
Aquatic Acute 1,	H400

Labelling:

Pictograms: GHS06, GHS05, GHS09

Signal word: Dgr

Hazard statements:

H301 + H311; Toxic if swallowed or in contact with skin.
H314; Causes severe skin burns and eye damage.
H317; May cause an allergic skin reaction.
H318; Causes serious eye damage.
H335; May cause respiratory irritation.
H400; Very toxic to aquatic life.

2.4.2 Current self-classification and labelling based on DSD criteria

Not applicable.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

2-Methylisothiazol-3(2H)-one is a biocidal active substance that has been reviewed according Regulation (EU) No. 528/2012 for use as a metalworking-fluid preservative (product type 13). In accordance with Article 36(2) of EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures such substance shall be subject to harmonised classification and labelling. A classification and labelling proposal based mainly on the information presented in the Competent Authority Report (CAR) for MIT.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

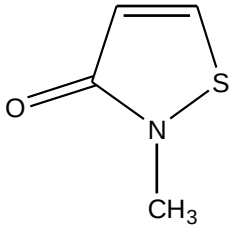
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	220-239-6
EC name:	2-methyl-2H-isothiazol-3-one
Common name, synonym:	2-methyl-2*H*-isothiazol-3-one MIT, MI, methylisothiazolinone, 2-methyl-4-isothiazoline-3-one,
Commercial name :	Kordek™ 573T Industrial Biocide, RH-573, Kordek ™ 573F, ACTICIDE® M 20, ACTICIDE® M 20 S, ACTICIDE® M 50
CAS number (EC inventory):	2682-20-4
CAS number:	2682-20-4
CAS name:	3(2H)-Isothiazolone, 2-methyl-
IUPAC name:	2-methylisothiazol-3(2*H*)-one
CLP Annex VI Index number:	/
Molecular formula:	C_4H_5NOS
Molecular weight range:	115.16 g/mol

Structural formula:



1.2 Composition of the substance

The active substance is manufactured by two applicants: Thor GmbH and Rohm and Haas. The active substance as manufactured from Rohm and Haas source is a solid technical grade active substance and from Thor GmbH source a technical concentrate (TK), 50 % MIT in water solution. Equivalence of both sources of active substance as manufactured according the criteria from TNsG on the assessment of technical equivalence was ascertained as there is a single assessment report, a single LOEP and a single set of specific provisions for the Union list of approved active substances. Substances from both sources are considered to have equivalent toxicity profile concerning the Tier II evaluation. The impurities are considered confidential and are therefore not given in this report. None of the impurities is considered relevant for classification purposes. There are no additives present in MIT.

1.2.1 Composition of test material

The minimum purity of 950 g/kg is applied for MIT. The minimum purity of 950 g/kg is supported by the analytical data (5-batch analysis) and it has been used in most of the toxicity and ecotoxicity tests in dossiers of Thor GmbH. A higher minimum purity, 980 g/kg, is supported by the 5-batch analysis and it has been used in most of the toxicity and ecotoxicity studies in the dossier of the Rohm and Haas.

1.3 Physico-chemical properties

Table 5: Summary of physico - chemical properties

Property	Value*	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Crystalline solid	1	Visual
Melting/freezing point	46.7 – 48.3 ºC	1	Directive 92/69/EC, A1 (Melting temperature devices with metal block)
	39 – 42.8 °C	1	OECD 102 (Capillary method according to Siwoloboff)
Boiling point	The active substance does not boil prior to decomposition. Decomposition starts at 235 °C.	1	ASTM E 537-86 (equivalent to EC method A2)
	The active substance does not boil prior to decomposition. Decomposition at about 236 °C.	1	OECD 103
Relative density	1.35	1	Method used is analogous to CIPAC MT 3.2. Pyknometer method
	1.39	1	OECD 109/ CIPAC MT 3/ Directive 92/69/EC, method A.3 (Pyknometer method)
Vapour pressure	0.73 Pa at 25 ºC (extrapolated) 0.408 Pa 20 ºC (extrapolated)	1	Directive 92/69/EC, A4 (Effusion method - vapour pressure balance)
	1.60 Pa at 25°C (extrapolated) 0.99 Pa at 20°C (extrapolated)	1	OECD 104 (Gas saturation method)
Surface tension	σ = 68.8 mN/m at 19.5 ºC(1 g/l solution)	1	Directive 92/69/EC, A5 (OECD harmonized ring method)
	σ = 72.32 mN/m at 20 ºC(1.01 g/l solution)	1	OECD 115, Directive 92/69/EC, A.5
Water solubility	> 1000 g/l	1	Directive 92/69/EC, A6 (Flask method)
	> 4287.2 g/l at pH = 4.5 and 20 °C	1	OECD 105 (Flask method)
Partition coefficient n-octanol/water	Kp = 0.326 (log Kp = -0.486) Temperature: 24 °C	1	OECD 107
	log Pow (1): pH 7: -0.34 (10 °C) pH 7: -0.32 (20 °C) pH 7: -0.34 (30 °C) pH 5: -0.26 (20 °C) pH 9: -0.28 (20 °C) log P_{OW} (2): -0.71 (20 °C)	1	OECD 117
Flammability	Not highly flammable.	1	Directive 92/69/EC, A10
Explosive properties	Not classified.	1	Test was not conducted, as the screening procedures applying structural examination, oxygen balance calculation and available thermodynamic data indicate that the active substance is not considered explosive.

Oxidising properties	Not classified.	1	Test was not conducted, as active substance has no functional groups capable of being significantly oxidizing.
Dissociation constant	Not applicable. MIT does not dissociate into ionic species.	1	-
	pK > 2.8 at 21 °C MIT may be considered as a low dissociated compound. With respect to the chemical structure MIT represents a weak base.	1	OECD 112 (Conductometer method)

* Values for two sources of the substance are available and are listed.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for classification and labelling.

2.2 Identified uses

MIT is widely used preservative (Product type 6 (In-can preservatives), 11 (Preservatives for liquid-cooling and processing systems), 12 (Slimicides) and 13 (Metalworking-fluid preservatives) according to Annex V of Regulation (EU) No. 528/2012).
MIT is a broad spectrum antimicrobial substance showing bactericidal, bacteristatic, fungicidal and fungistatic function. MIT exhibits rapid inhibition of growth at very low levels and cidal effects at higher levelsor for longer contact periods. MIT is most active as a bacteriocide whereas the antifungal activity of MIT is shown at higher use levels.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 6: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
See Table 5			

3.1 Physico-chemical hazards

No classification is required.

3.1.1 Summary and discussion of physico-chemical properties

The physico-chemical properties of 2-methylisothiazol-3(2H)-one were assessed in the Slovene's Competent Authority Report (CAR) regarding Regulation (EU) No. 528/2012 and shall be included in Union list of active substances approved for use in biocidal products. Based on the result of the test data 2-methylisothiazol-3(2H)-one is not explosive, oxidising, flammable or auto-flammable. The MIT can be considered as thermally stable at room temperature. No flash point was determined as the substance is a solid, and does not have a melting point below 40 °C. There are no known incompatible packing materials. The summaries included in this

proposal are copied from the CAR. For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarised in Table5.

3.1.2 Comparison with the CLP classification criteria

Not relevant.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of methylisothiazolinone (MIT) were assessed in the Slovene's Competent Authority Report (CAR) regarding Regulation (EU) No. 528/2012 and shall be included in Union list of active substances approved for use in biocidal products.

The summaries included in this proposal are copied from the CAR. For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarisedsummarised in a table. Detailed information is only included for the key studies used to derive the classification. References to individual studies are not included. For more details the reader is referred to the CAR.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetics of MIT has been investigated *in vivo* in rats and mice by the oral route. Toxicokinetics of MIT by the dermal route has been studies in rats *in vivo* and *in vitro* (see Table 7).

4.1.2 Human information

The toxicokinetics of MIT has been investigated *in vitro* in human skin by the dermal route (see Table 7).

4.1.3 Summary and discussion on toxicokinetics

Table 7: Summary of toxicokinetics, metabolism and distribution studies

Route	Species	Method Guideline	Label	Dose level	Analysed parameters	Reference
Oral	Rat, Sprague-Dawley, 3-4 /sex/group	OECD 417 GLP	4,5-^{14}C-MIT, radiochemical purity 99.08 %	Single dose: 5 and 50 mg/kg bw	Absorption, distribution, metabolism, elimination	A6.2/04 (Rohm and Haas)
Oral	Rat/Sprague Dawley, 4 females/ group	OECD 417 GLP	4,5-^{14}C-MIT, radiochemical purity 96.90 %	Single dose: 50 mg/kg bw	Metabolism	A6.2/05 (Rohm and Haas)
Oral	Mouse/ CD-1, 15/sex/group	No; Study conducted to support MN test result GLP	^{14}C-MIT, radiochemical purity 96.70 %	100 mg/kg bw; exposure period 1, 3, 6, 24 and 48 hours	Distribution	A6.2/03 (Rohm and Haas)
Oral	Rat, Sprague-Dawley, 4 /sex/group,	OECD 417 GLP	4,5-^{14}C-MIT, radiochemical purity 98 %	Single dose: 50 mg/kg bw	Absorption, distribution, metabolism, elimination	A 6.2-01 (Thor GmbH)
Oral	Rat/Sprague Dawley, 4 females/ group	OECD 417 GLP	4,5-^{14}C-MIT, radiochemical purity 96.90 %	Single dose: 50 mg/kg bw; samples from the study A 6.2-01 (Thor GmbH) were analysed	Metabolism	A6.2-02 (Thor GmbH)
Dermal (*in vitro*)	Rat	OECD draft guidelines for dermal absorption *in vitro*, GLP	^{14}C-MIT, radiochemical purity 99.88 %	25, 75 and 150 ppm MIT in water (equal to 0.66, 1.97 and 3.97 µg/cm^2)	Absorption	A6.2/01 (Rohm and Haas)
Dermal (*in vitro*)	Human epidermis	OECD 428 GLP	4,5-^{14}C-MIT, radiochemical purity 96.90 %	52.2, 104.3 and 313 µg MIT/ml, aqueous solution, 100 µg/ml in CTAE shampoo formulation, body lotion formulation and facial cream	Absorption	A6.2/02 (Rohm and Haas)

Dermal (*in vivo*)	Rat/ Sprague Dawley, 2/time of sacrifice	Non-guideline study No	^{14}C-MIT, radiochemical purity > 98 %	0.2 %dilution of MIT; exposure period 24 hours	Absorption	A6.2-03 (Thor GmbH)

A study to investigate the oral absorption of 4.5-^{14}C-MIT has been conducted in rats. Male and female rats (3-4/sex) were dosed by single oral gavage with 5 and 50 mg/kg bw radiolabelled MIT and then placed individually in metabolism cages. Urine, faeces and cage wash were collected at 24 h intervals over a 96-hour period post-dosing. In separate group blood and plasma were collected at 1, 3, 6, 24, 72 and 96 hours post-dose. MIT was excreted rapidly from the rat; 80-87 % of the administered dose was eliminated within 24 hours; a majority of the radioactivity was recovered in urine and cage rinse (53-70 %) and a lesser amount was recovered in feces (21-37 %). After 96 h tissues contained 1.9-3.6 % of dosed radioactivity which was predominately located in the blood. Total mean recovery of radioactivity ranged from 92-96 %. Radioactivity ratio detected in the urine, cage wash and tissues was considered absorbed. This means that at high dose 55-58 % of MIT was absorbed in females and males, respectively, and 67-73 % in females and males at the low dose.

In both sexes, t_{max} in blood and plasma was reached at 1 h post-dose in animals exposed to 5 mg base-equivalents/kg. T_{max} was 1.7 h in males and 3 h in females in 50 mg base-equivalents/kg groups. The elimination half-lives of ^{14}C-label from plasma ($T_{1/2}$ initial) were rapid and ranged from 3.2-3.85 h in low-dose group and 5.1-6.2 h in high dose group.

MIT was extensively metabolized in the rats. Twenty-three radioactive components were observed in urine and feces samples from the HPLC radioprofiling. Among these N-methyl malonamic acid and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl propionamide were detected as the major components in the urine (21-23 % and 10-23 % of the dose, respectively). N-methyl-3-hydroxyl-propionamide was also detected in urine at levels in the range of ~4 % to 5 % of the dose. M2 contained at least three components and was the major component detected in the feces. Other metabolites designated as M9-A/M9-B were proposed as mercapturate conjugates. Parent compound was not detected in either urine or feces samples. All metabolites accounting for >1 % of the administered dose were identified and/or characterized by LC/MS and LC/MS/MS. The metabolites of MIT are comprised of a variety of Phase I metabolites consisting of reductive and oxidative cleavage products of MIT and Phase II metabolites consisting of mercapturic acid conjugates of Phase I metabolites of MIT (see Figure 3.1.1). The formation of mercapturic acid conjugates from MIT in rats was supported by the finding of many glutathione conjugates and related conjugates in rat bile from bile-duct cannulated rat metabolism study of MIT (Rohm and Haas).

A second study to investigate the metabolism of 4.5-^{14}C-MIT has been conducted in female rats. Following single gavage administration of 50 mg/kg bw bile, urine, feces and cage wash were collected after 24 hours. During the 24 h, an average of 29.09 % of the administered dose was excreted in bile, 52.92 % in urine and cage wash and 6.14 % in feces. Total recovery of the administered dose in bile, urine and feces averaged 88.16 %. Twelve radioactive components were observed in urine and feces samples from the HPLC radioprofiling. N-Methyl malonamic acid and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide were detected as the major components in the urine (~23 % and ~9.5 % of the dose, respectively). Twenty radioactive components were observed in the bile sample from the HPLC radioprofiling, each accounting for <5 % of the dose. Only glutathione conjugate of 3-thiomethyl-N-methyl-propionamide accounted for 4.9 % of the dose. The initial HPLC radio-chromatography

revealed the presence of at least 31 components derived from MIT. All metabolites accounting for >1 % of the administered dose, and some minor ones, accounting for less than 1 %, were identified and/or characterized by LC/MS and LC/MS/MS. No parent compound was detected in urine, feces or bile. MIT was metabolized to a variety of Phase I metabolites consisting of reductive and oxidative cleavage products of MIT and Phase II metabolites consisting of glutathione or glutathione derived conjugates of Phase I metabolites of MIT (see Figure 3.1.2). In addition to glutathione conjugates, di-conjugates with glucuronic acid were also found in bile. Following a single oral dose exposure of female bile-cannulated rats 53 % MIT was absorbed (urine and cage rinse) (Rohm and Haas).

A study on tissue distribution of MIT in mice was conducted to support the *in vivo* mouse micronucleus assay. Mice were exposed to 100 mg/kg bw by gavage. Animals were sacrificed 1, 3, 6, 24 and 48 hours post-dose and radioactivity residues were determined in blood, plasma, liver, femur bone and bone marrow. Several animals were ill after treatment and 5 died before the scheduled termination. High radioactivity values were found in all tissues at the earlier time points, with the liver being the highest (107 ppm in male 1 hour sample; 56.5 ppm in female 1 hour sample), and bone being the lowest (27.0 ppm in male 1 h sample; 18.1 ppm in female 1 h sample). After 24 hours, radioactive residues in the tissues declined significantly and ranged from 0.510 to 7.50 ppm in male tissues and from 0.295 to 9.00 ppm in female tissues. The tissue to plasma ratio showed that radioactivity partitioned preferentially from plasma to tissues after 24 hour post-dose. Blood had the highest tissue to plasma ratio at 48 hour post-dose in both male and female mice. Mean concentrations of radioactive residues in bone marrow ranged from 1.16 to 39.4 ppm in males and 1.06 to 30.4 ppm in females over the 48 hour period. In general, male tissues appeared to have higher radioactive residues than female tissues (Rohm and Haas).

Absorption, distribution, metabolism and excretion of (^{14}C)-MIT was investigated once more in rats exposed to a single oral dose of MIT by gavage. Following the oral dose of (^{14}C)-MIT at a nominal dose level of 50 mg/kg bw 93.6% and 94.0% of the administered radioactivity was recovered in males and females, respectively, after 7 days. Absorption and excretion of radioactivity was rapid; in the first 24 hours 89.1 % and 79.5 % of MIT were excreted in males and females respectively, when combining pilot and main study data. The major proportion of the dose was excreted in urine (66 % and 54 % in males and females, respectively, in the first 24 hours). An indication of the rapidity of absorption can be gained from the fact that up to 72% of the urinary radioactivity was collected within 6 h of dose administration. Faecal elimination was also an important route of excretion with 24.5% (male) and 27.4% (female) of the administered radioactivity recovered at 168 h. Radioactivity in expired air accounted for < 0.1% of the dose indicating that metabolism to $^{14}CO_2$ is not an important route of excretion. Radioactivity was detected in all tissues at 168 h following dose administration. The carcass also contained ca 2% of the administered dose. MIT was detected in blood, bone, brain, fat, heart, lung, spleen, liver kidneys, gonads, muscle and adrenals. Radioactivity was not detected in plasma but blood contained the highest concentrations of radioactivity of any of the tissues sampled, indicating that radioactivity was binding to the red blood cells. For a total blood volume in the rat of cca 15 ml, this equates to ca. 2% of the administered radioactivity still being present at 168 h after dose administration. This may also account for the high levels of radioactivity in highly vascularised tissues. Total recovery in this study was 94 % for males and females. Radioactivity ratio detected in the urine, cage wash, cage debris and tissues was considered absorbed. This means that 67-69 % of MIT was absorbed in males and females.

The test article was extensively metabolised with no evidence for parent compound in either urine or faeces. The metabolite profiles showed there to be no sex differences in the metabolism of either test article. LC-MS analysis of concentrated urine proved to be inconclusive partly due to the numerous metabolites present at fairly low levels and partly due to high levels of co-eluting endogenous material in the sample which obscured the spectra (Thor GmbH).

The metabolism of MIT was mediated by glutathione conjugation. Structures were assigned for the three major urinary metabolites of MIT and tentative structures were proposed for two minor isometric metabolites. These metabolites accounted for 58.9% and 55% of the administered radioactivity in male and female animals, respectively. A minor metabolite remained unidentified but it accounted for only a maximum of 7.0 % of the dose.

Faecal metabolites following the administration of (^{14}C)-MIT, accounting for 24.5 and 27.4% of the dose were not further characterised as the metabolite profiles indicated the presence of numerous components. The most abundant of the metabolites accounted for less than 6.4% of the dose. A metabolic pathway was proposed for MIT in rat (Figure 3.1.3) (Thor GmbH).

Figure 3.1.1 Proposed metabolic pathway of MIT

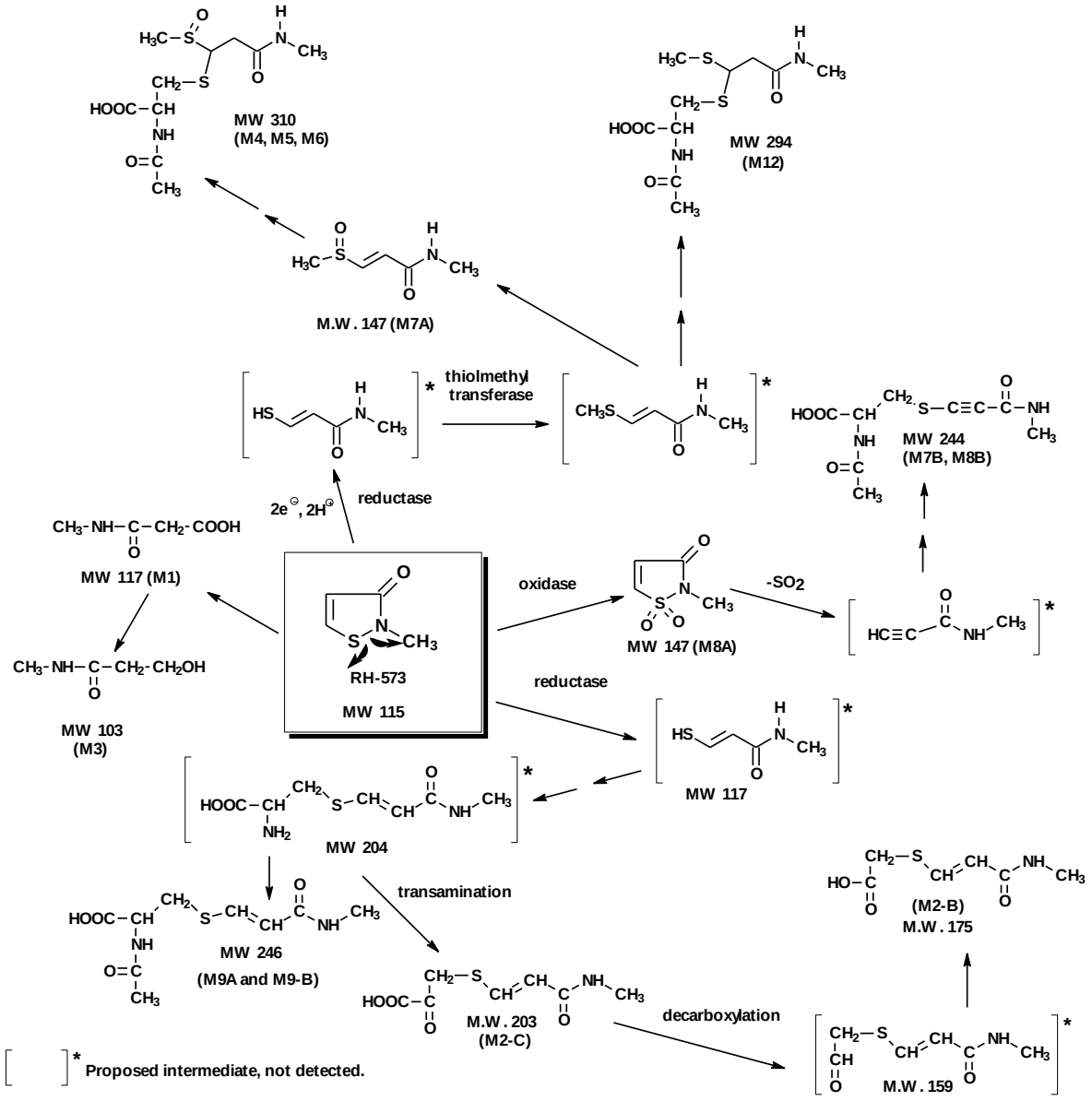


Figure 3.1.2 Proposed metabolic pathway of MIT (study with bile cannulated rats)

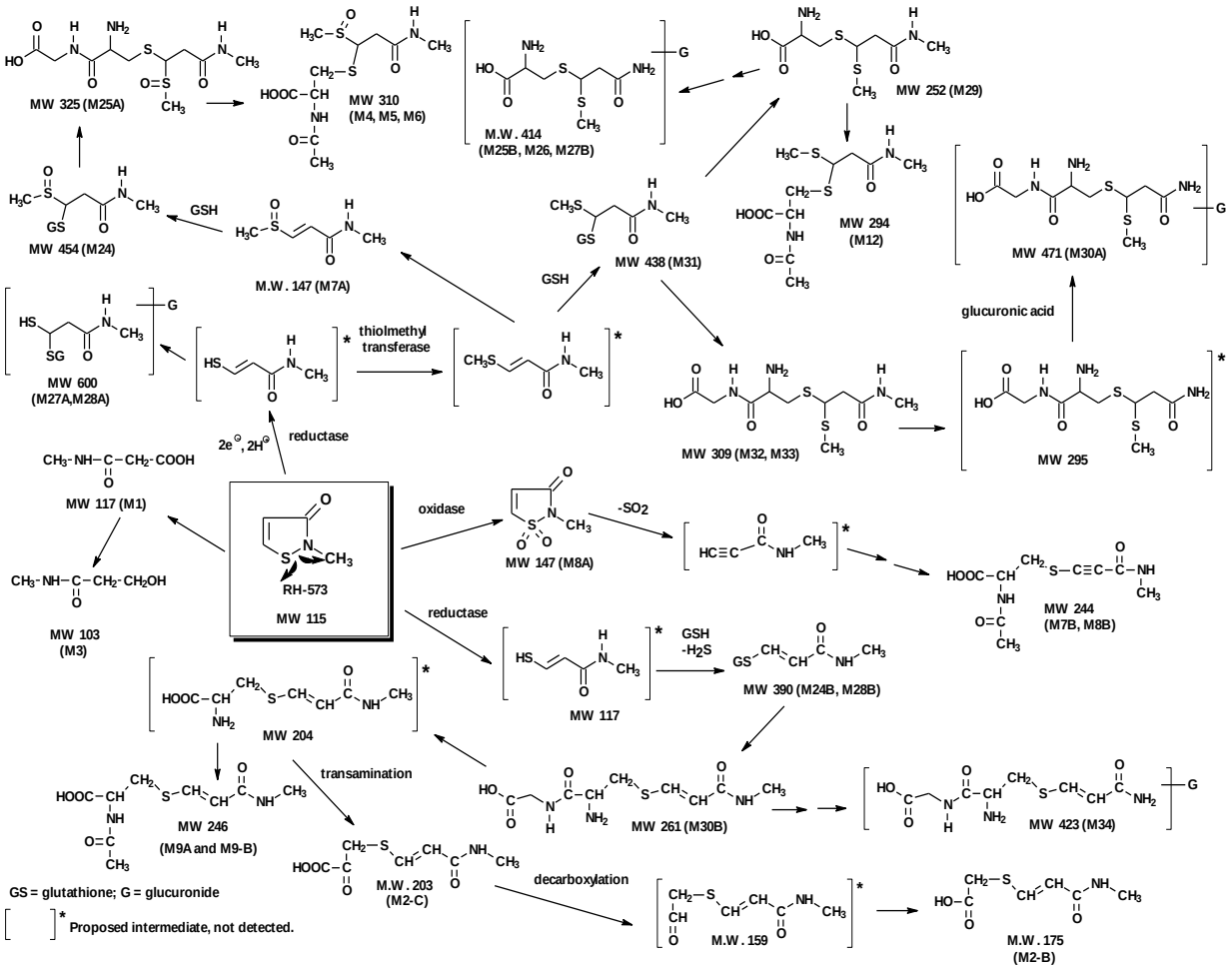
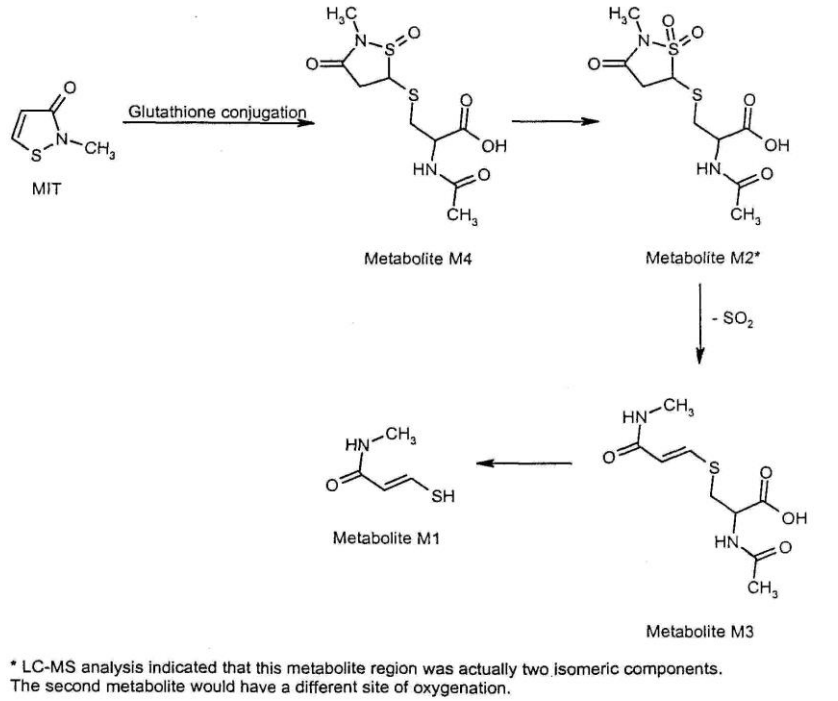

MW 325 (M25A)
MW 310 (M4, M5, M6)
M.W. 414 (M25B, M26, M27B)
MW 252 (M29)
MW 454 (M24)
GSH
M.W. 147 (M7A)
MW 294 (M12)
MW 438 (M31)
MW 471 (M30A)
glucuronic acid
MW 600 (M27A,M28A)
thiolmethyl transferase
MW 309 (M32, M33)
MW 295
2e⊖, 2H⊕
reductase
MW 117 (M1)
oxidase
MW 147 (M8A)
-SO2
RH-573
MW 115
MW 244 (M7B, M8B)
MW 103 (M3)
reductase
GSH -H2S
MW 117
MW 390 (M24B, M28B)
MW 204
transamination
MW 246 (M9A and M9-B)
MW 261 (M30B)
MW 423 (M34)
M.W. 203 (M2-C)
decarboxylation
M.W. 159
M.W. 175 (M2-B)
GS = glutathione; G = glucuronide
Proposed intermediate, not detected.

Figure 3.1 Proposed metabolic pathway for MIT in the rat



Dermal absorption

Two *in vitro* dermal absorption studies on MIT have been conducted with human skin epidermis and dermatomed rat skin (Rohm and Haas) and one *in vivo* dermal absorption study was performed in rats (Thor GmbH).

In the first study on human epidermis aqueous solutions of MIT (52.2 µg/ml, 104.3 µg/ml and 313 µg/ml), MIT in CTEA shampoo formulation (100 µg/ml), MIT in body lotion formulation (100 µg/ml) and MIT in facial cream (100 µg/ml) were applied for 24 hours under occlusion. After exposure remaining MIT was washed off the skin. Donor chamber, receptor fluid, stratum corneum, epidermis and skin wash were analysed for the presence of radioactivity. MIT readily penetrated human skin; 29.8, 38.0 and 54.7 % of MIT were detected in the receptor fluid after application of 52.2, 104 and 313 µg/ml, respectively. When including ^{14}C-label retained in the epidermis and lower layers of stratum corneum (tape strips 3-5), 65.5, 62.0 and 67.3% of the applied dose was 'potentially' systemically available at MIT concentrations of 52.2, 104 and 313 µg/ml, respectively.

When MIT (100 µg/ml) was formulated in a shampoo, body lotion and facial cream, 29.5, 9.0 and 19.6 % of the applied dose was absorbed across the epidermis (24 h, occluded exposure), respectively. When including ^{14}C-label retained in the epidermis, epidermis and lower layers of stratum corneum, 52.3, 27.8 and 37.3% were absorbed through human skin from the shampoo formulation, body lotion formulation and facial cream.

Dermal absorption of MIT in aqueous solution was similar at all concentrations of MIT in aqueous solutions tested (62 - 67 %).

In the second study, MIT in aqueous solution at 25, 75 and 150 ppm was applied on dermatomed rat skin. After 24 hours excessive MIT was wiped off the skin. Radioactivity in tape strips, epidermis, dermis and wipe-offs was determined. During the 24 hr exposure period 21.4, 33.7 and 51.2 % of the dose appeared in the receptor fluid following exposure to 25, 75 and 150 ppm MIT, respectively. Majority of the ^{14}C-label was located in epidermal sections (29.2-46.4 % of dose) of skin and smaller amounts of ^{14}C-label were located in the stratum corneum (3.8-10.4 %) and dermis (0.2-0.9 % of dose). According to the Guidance Document on Dermal Absorption (SANCO, ver. 7) radioactivity in the skin (except the first two tape strips) should be considered absorbed. Therefore MIT in receptor fluid, dermis and epidermis was considered absorbed and over the range of concentrations tested, 25, 75 and 150 ppm active ingredient, 68, 68.8 and 81.3 % of applied dose were absorbed across the skin barrier following a 24 h exposure period.

In the dermal penetration study *in vivo* in male rats MIT was not tested alone, but in combination with CMIT. Test material Kathone 886 contains 14 % of CMIT/MIT mixture in ratio 3:1. This study was not conducted in compliance with any guideline or GLP. Two animals were used for each time point (24, 48 and 96 hours) and they were exposed to 0.2 ml of tested substance (2000 ppm active ingredient or 0.8 mg/kg bw). Test substance was applied within a glass ring glued to the shaved skin of the rat and covered by a porous top. After 24 hours residues on skin were removed by cotton swabs moistened with water. Excretions, blood, skin wash, ring wash, skin at the application site, testicles and remaining carcass were analysed for radioactivity after 24, 48 and 96 hours. Recovery ranged from 82-91 % of applied dose of MIT.

A major part of the applied dose (36-65 %) remained in the skin after washing. However the amount decreased by 29 % and 22 % of applied dose from 24 and 48 hours to 96 hours, respectively. Total absorption (excretions, blood, testes, remaining carcass) increased from 8.9 % of applied dose at 24 hours after application to 16.1 % and 23.9 % at 48 and 96 hours after application, respectively. The fact that at least urinary excretion did not relevantly increase between 72 and 96 hours, and as residues in testes and carcass decreased between 48 and 72 hours indicates that the amount remaining to be associated with the treated skin after 96 hours may not be available for further systemic absorption.

The fact that the increase in absorption between 24 hours and the two later sacrifice points only corresponds to 1/3 to 1/2 of the decrease in skin residues at the same time period indicates that maximum 1/2 of the skin depot remaining at 96 hours may be available for further systemic absorption.

Including the considerations on the skin depot the worst-case total absorption of MIT is therefore considered to be 41.7 % of applied dose corresponding to the sum of the absorbed dose (23.9 %) and 1/2 of the skin depot at 96 hours (17.8 %). Despite deficiencies in the study we consider this value conservative enough because dermal penetration through rat skin is usually higher compared to human skin.

Conclusions

The absorption of MIT from rats treated with 5-50 mg base-eq. MIT/kg bw was 92-96 %. Absorption and excretion were rapid, with 80-87 % of ^{14}C label excreted in 24 hours. MIT was distributed to blood, plasma, liver, femur bone and bone marrow tissues following a single oral

dose (100 base-equivalents/kg bw) of the test material to adult male and female mice. **There was no evidence of accumulation of MIT in the animal body.** MIT was extensively metabolised in rat, with N-methyl malonamic acid and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl propionamide being the major components in the urine. Parent compound was not observed in urine, feces or bile.

In the second toxicokinetic study of MIT similar results were obtained after single oral dose 50 mg (^{14}C)-MIT /kg bw. In male and female rats 93.6% and 94.0% of the administered radioactivity was recovered after 7 days, respectively. The absorption and excretion within 24 hours were 89.1 % and 79.5 % of administered dose. Parent compound was not observed in excretions. Also in this study MIT was widely distributed to blood, bone, brain, fat, heart, lung, spleen, liver kidneys, gonads, muscle and adrenals. Radioactivity was not detected in plasma but blood contained the highest concentrations of radioactivity of any of the tissues sampled, indicating that radioactivity was binding to the red blood cells. MIT was extensively metabolised to numerous metabolites and glutathione conjugation is involved in its metabolism. **No evidence of accumulation in the body was observed.**

To conclude, at 50 mg/kg bw 55-58 % of MIT was absorbed in the first study and 67-69 % in the second study. In bile-cannulated rats that received 50 mg/kg bw 53 % of MIT was absorbed. In rats treated with 5 mg/kg bw 67-69 % of MIT was absorbed. For the risk assessment 55 % will be used as a value for the oral absorption of MIT, representing the worst case.

No information is available on inhalation absorption of MIT so the default value of 100 % will be used in the risk assessment.

When dermal absorption of MIT was tested through human skin 65.5, 62.0 and 67.3 % of the applied dose was 'potentially' systemically available at MIT concentrations of 52.2, 104 and 313 µg/ml (in water), respectively. When MIT (100 µg/ml) was formulated in a shampoo, body lotion and facial cream 52.3%, 27.8 % and 37.3% were absorbed through human skin from the shampoo formulation, body lotion formulation and facial cream, respectively.

Dermal absorption of MIT was tested also on rat dermatomed skin at concentrations 25, 75 and 150 µg active ingredient /ml (in water) where 68 %, 68.8 % and 81.3 % of applied dose were absorbed across the skin barrier following a 24 h exposure period. Higher penetration of rat skin compared to humans was expected.

In *in vivo* study in rats 41.7 % of the applied MIT could become systemically available. The *in vivo* study was not conducted according to a guideline and GLP and at one observational period data from only one animal was used.

Dermal absorption study on human epidermis is the most appropriate to determine an overall dermal absorption value for MIT in MWF. In the risk assessment the average dermal absorption value of 67 % will be considered for MIT in MWF. This value has been determined for the MIT in aqueous dilution at concentrations in the same range as proposed to be used in MWF. Even though the MIT dermal absorption value of 67 % was determined for water solution and not for MWF we believe that it is conservative enough and can be used for the exposure estimate of MWF.

Since MIT is corrosive or irritant it is likely to induce skin damage that alters skin penetration. Therefore 100 % dermal absorption should be used for the risk assessment of the concentrate and biocidal product (containing 20 or 50 % of the active substance as the representative products for the inclusion on Union list of active substances approved for use in biocidal products according Regulation (EU) No. 528/2012.

4.2 Acute toxicity

The acute toxicity of MIT has been investigated by the oral (rat, mouse), dermal (rat) and inhalation (rat, mouse) route.

Table 8: Summary table of relevant acute toxicity studies

Route	**Method/ Guideline**	**Species/Strain/Sex no/group**	**Tested material/ Dose levels / duration of exposure**	**Value LD_{50}/lC_{50}**	**Reference**
Oral gavage	OECD 401 GLP	Rat/ Crl:CD®BR, 6/sex/dose	RH-573 Technical (purity 99.7% a.i.) 75, 150, 180, 225 and 300 mg MIT/kg bw 14d post-exposure period	LD_{50} = 235 mg MIT/kg bw, males LD_{50} = 183 mg MIT/kg bw, females	A6.1.1/01 (Rohm and Haas)
Oral gavage	EPA 40 CFR 158.340 GLP	Rat/ Crl:CD®BR, 6/sex/dose	Kordek 573F (50 % MIT in water) 150, 180, 225 and 300 mg MIT/kg bw 14d post-exposure period	LD_{50} = 232 to 249 mg MIT/kg bw, males LD_{50} = 120 mg MIT/kg bw, females	A6.1.1/02 (Rohm and Haas)
Oral gavage	OECD 401 GLP	Mice/ Crl:CD-1® (ICR)BR, 6/sex/dose	Kordek ™573T (purity 97.5% a.s.) 150, 200 and 250 mg MIT/kg bw 14d post-exposure period	LD_{50} = 167 mg MIT/kg bw	A6.1.1/03 (Rohm and Haas)
Oral gavage	OECD 401 GLP	Rat/Wistar, 5/sex/dose	ACTICIDE SR 3267* (purity 49.0% a.s.) 225, 338, 506, 759 and 1139 mg Acticide SR 3267 /kg bw; equal to 110.3, 165.6, 247.9, 371.9 and 558.1 mg MIT/kg bw 14d post-exposure period	LD_{50} = 328 mg MIT /kg bw, males 247 mg MIT/kg bw, females	A 6.1.1-01 (Thor GmbH)

Dermal	OECD 402 GLP	Rat/ Crl:CD®BR, 6/sex/dose	Kordek ™573T (purity 97.5% a.s.) 100, 200, 300 (males only) and 400 mg (males and females) MIT/kg bw 14d post-exposure period	LD_{50} = 242 mg MIT/kg bw	A6.1.2/01 (Rohm and Haas)
Dermal	OECD 402 GLP	Rat/Wistar, 5/sex/dose	ACTICIDE SR 3267 (purity 49.0% a.s.) 4082 mg Acticide SR 3267 /kg bw; equal to 2000 mg MIT/kg bw 14d post-exposure period	LD_{50} > 2000 mg MIT/kg bw	A6.1.2-01 (Thor GmbH)
Inhalation	OECD 403 GLP	Rat/ Crl:CD®BR, 6/sex/dose	RH-573 Technical, aerosol (purity 97.8% a.s.) 2.09, 1.07, 0.15, 0.012 and 0.046 mg MIT/l air, 4 hours Nose-only exposure 14d post-exposure period	4hr LC_{50} = 0.11 mg MIT/l air	A6.1.3a/01 (Rohm and Haas)
Inhalation	OECD 403 GLP	Rat/ Crl:CD®BR, 5/sex/dose	Kordek 573F (50 % MIT in water) (purity 53.52% a.s. in water) 0.15, 0.25, 0.47 and 0.68 mg MIT/l air, 4 hours Nose-only exposure 14d post-exposure period	4hr LC_{50} = 0.19 mg MIT/l air	A6.1.3a/02 (Rohm and Haas)
Inhalation	OECD 403 GLP	Rat/ Crl:(Wi) Br, 5/sex/dose	ACTICIDE SR 3267, aerosol (purity 49.8% a.s.) 0, 0.086, 0.173 and 0.327 mg Acticide SR 3267/l air, 4 hours; equal to 0.042, 0.086 and 0.163 mg MIT/l Nose-only exposure 14d post-exposure period	4hr LC_{50} = 0.134 mg MIT/l air	A6.1.3-01 (Thor GmbH)

*Acticide SR 3267 - 49 % MIT in water.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Acute oral toxicity studies were performed on rat and mouse. Clinical signs observed in treated animals were passiveness, ataxia, lethargy, diarrhea or soft feces, scant or no feces, yellow or brown stained anogenital area, soiling and wetness in anogenital area, red-stained muzzle and lacrimation, piloerection and ptosis. Necropsies of the descendents revealed gastrointestinal changes. In the survivors no gross changes were observed. Female rats were more sensitive than males. **Lowest acute oral LD_{50} was 120 mg MIT/kg bw in female rats (Rohm and Haas).**

Another oral toxicity study was performed in rats. Mortalities occurred at 247.9, 371.9 and 558.1 mg/kg bw on day of exposure and two following days. The following clinical signs were observed in the study: piloerection, crouching, occasional lethargy and tremor. Surviving animals recovered copletely on day 3 of testing. Body weights of the surviving animals increased. Necopsy findings in descendents and survivors revealed mucous membrane of the stomach reddened by bloody/aqueous secretion or a detachment of mucous lining, the stomach appeared well contracted. In some cases liquid gastric content was detected. Reddened intestines were also observed. These findings are in line with irritant nature of MIT. Acute oral LD_{50} was 328 mg MIT/kg bw for males and 247 mg MIT/kg bw (or 669 mg Acticide 3267/kg bw in male and 504 mg Acticide 3267/kg bw in female rats) (Thor GmbH).

4.2.1.2 Acute toxicity: inhalation

In acute inhalation toxicity studies the following clinical signs were observed: gasping, rales, labored breathing, respiratory noise, salivation, red stained muzzle and eyes, nasal exudate, ataxia, passiveness, prostration, arched back and unkempt fur. 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. **The lowest acute inhalation LC_{50} was 0.11 mg MIT/l air (Rohm and Haas).**

In acute inhalation toxicity study of Acticide SR 3267 mortalities were observed at 0.173 and 0.327 mg/l air on day of exposure and day 1. Animals exposed to 0.086 mg MIT/l air 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 MIT/l air 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 second and three days of observation period.

In animals exposed to 0.327 mg MIT/l dyspnoea and laboured breathing occurred from the 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 than those exposed to 0.086 mg/l and became symptom-free on the third day of the observation period. After symptom subsided animal's behaviour and general state during the remaining period of observation was normal in all dose groups. LC_{50} for males was determined to be 0.148 mg MIT/l air and for females 0.124 mg MIT/l air after 4 hours of exposure. Results of this study indicate that MIT is respiratory irritant.

4.2.1.3 Acute toxicity: dermal

Acute dermal toxicity of MIT was tested in rats. Clinical signs in treated animals included scant or no feces, passiveness and ataxia. Body weight in the survivors was decreased compared to controls. Effects on skin persisted until study termination on day 14; blanching, edema, darkened areas, eschar, sloughing, scabbed areas and desiccation. Necropsy of the descendents revealed gastrointestinal changes. In the survivors no gross changes were observed. Female rats were more sensitive than males. **Acute dermal LD_{50} 242 mg MIT/kg bw was determined in male rats (Rohm and Haas).**

A second acute dermal toxicity study in rats is available, with reported LD_{50} value >2000 mg MIT/kg bw (or 4082 mg Acticide 3267/kg bw). No mortalities and no clinical signs of intoxication were observed in this study. After dermal treatment in 5 of 5 male and 4 of 5 female animals moderate to severe erythema and very slight to slight oedema were seen. Later the area of application was scabby in all animals and at study termination eschar was formed. Body weight of males increased during the observation time but no body weight increase was recorded in female animals after the application. In acute dermal toxicity study strong irritation of skin was observed and no systemic toxicity (Thor GmbH).

Results of two acute dermal toxicity studies differ, but based on study summaries and study reports there is no clear reason for such difference. **However, the proposal for classification of MIT regarding acute dermal toxicity is based on more conservative study.**

4.2.1.4 Acute toxicity: other routes

No data available

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

In acute oral toxicity studies mortalities, clinical signs and necropsy findings (reddened mucous membrane of the stomach and reddened intestines) were observed. MIT was found to be toxic **by oral route.**

In one acute dermal toxicity study the exposure to MIT induced mortalities, skin blanching, edema, darkened areas, eschar formation, sloughing of skin and gastrointestinal changes observed at the necropsy. Second acute dermal toxicity studyshowed no effect after contact of MIT with skin. Taking into account the worst case (more conservative result) it is **acutely toxic by dermal route.**

In all acute inhalation toxicity studies MIT caused mortalities, laboured breathing, dyspnoea, increased respiration rate, reddish discharge around the nose and redness of lung lobes and red pin point foci on the lungs of rats. Signs of respiratory irritation were observed in these studies. MIT it is fatal **by inhalation route.**

4.2.4 Comparison with CLP classification criteria

Acute oral toxicity: MIT shall be classified as **Acute Tox. 3; H301** (Toxic if swallowed) on the basis of the lowest LD_{50} **120 mg MIT/kg bw** (female rat), because this LD_{50} is within the limits 50 mg/kg < LD_{50} ≤ 300 mg/kg.

Acute dermal toxicity: MIT shall be classified as **Acute Tox. 3; H311** (Toxic in contact with skin) on the bases of the lowest LD_{50} **242 mg MIT/kg bw** (female rat), because this LD_{50} is within the limits 200 mg/kg < LD_{50} ≤ 1000 mg/kg

Acute inhalation toxicity: MIT shall be classified as **Acute Tox. 2; H330 (**Fatal if inhaled) on the bases of the lowest LD_{50} **0.11 mg MIT/l air** (rat), because this LD50 is within the limits 0.05 mg/l /4h < LD_{50} ≤ 0.5 mg/l/4h.

4.2.5 Conclusions on classification and labelling

In accordance with the provisons of CLP Regulation (EC) No 12272/2008 MIT shall be assigned with pictogram **GHS06,** with signal word "Danger" and with the following hazard statements: **H301** (toxic if swallowed), **H311** (Toxic in contact with skin) and **H330 (**Fatal if inhaled).

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

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

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

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

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

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

Comments received during public consultation

Oral toxicity

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

Dermal toxicity

One manufacturer and one MSCA considered Acute Tox. 3 to be appropriate for MIT. A second manufacturer disagreed with the proposal for Category 3. The company considered that the large discrepancy in the LD_{50} values between the 2 studies (242 mg /kg vs 2000 mg/kg) could be explained by the different states of aggregation of the active substances that were tested in the two different studies. The first study (LD_{50} = 242 mg/kg bw) used the solid/neat substance (97.5%) wetted with vehicle, whereas the

second study (LD_{50} > 200 mg/kg bw) used a technical watery solution (49% a.i.). On this basis, the manufacturer suggested a "split entry classification" to recognise the two different conditions of aggregation of MIT leading to different results in respect of dermal toxicity.

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

Inhalation

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

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

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

EUH 071

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

Assessment and comparison with the classification criteria

Oral

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

Dermal

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

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

Inhalation

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

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

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

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

4.3 Specific target organ toxicity – single exposure (STOT SE)

In acute inhalation toxicity studies in rats clinical signs indicating respiratory irritation were observed, e.g. gasping, rales, labored breathing, respiratory noise, red-stained eyes and muzzle, and nasal exudate (Doc IIA: A6.1.3a/01, A6.1.3a/02, A6.1.3-01). In all acute inhalation studies (Doc IIA, A6.1.3a/01) 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 and signs of point-like hemorrhages on the lungs. In one study pulmonary emphysema was also observed in treated animals. Due to the corrosive nature of MIT and since the effects were observed on the lungs, MIT should additionally be considered as corrosive to respiratory tract.

Respiratory irritation of MIT was tested in upper airway irritation test in mice (Doc IIA, A6.1.3b/01) and result is presented in Table 9.

Table 9: Summary of respiratory irritation data

Route	**Method/ Guideline**	**Species/Strain/Sex no/group**	**Tested material/ Dose levels / duration of exposure**	**Value RD_{50}**	**Reference**
Upper airway irritation potential	ASTM E981-84 GLP	Mice/Swiss Webster derived Crl:CFW® (SW)BR/ 4 males	RH-573 Technical (purity 98.6% a.s.) 3.12, 6.67, 10.5, 27.8, 64.6, 74.9, 90.7, 92.2 and 157 µg MIT/l, 10 minutes Head only exposure 15 minutes post-exposure period	RD_{50} > 157 µg MIT/l	A6.1.3b/01 (Rohm and Haas, Thor GmbH)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

MIT is a corrosive substance and can therefore be considered as respiratory irritant as indicated by the acute inhalation toxicity study in rats. In addition, the upper airway irritation of MIT was evaluated in mice and RD_{50} > 157 µg/l was determined. Respiration rate was decreased on 47 %. According to the used guidance MIT would be rated as moderate sensory irritant (20-50 % decrease in respiration rate). The upper airway irritation test is a measure of sensory irritation and is commonly used for setting up workplace exposure limits, but not for classification purposes.

However results from acute inhalation toxicity studies in rats, supported by an upper airway irritation test in mice and considering the corrosive properties of MIT, indicate that MIT should be classified as STOT SE 3, H335. But corrosivity of MIT is covered by the classification Skin Corr., because this is considered to be the mechanism of pulmonary toxicity. Therefore MIT shall be labelled as EUH071

4.3.2 Comparison with CLP classification criteria

CLP:

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure should be classified as STOT SE 1 or 2 according to the CLP Regulation. Classification should be supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect that clearly impacts health. Classification in STOT SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

MIT shall be classified as STOT SE 3, H335 (May cause respiratory irritation) on the bases of the clinical signs and necropsy findings observed in acute inhalation toxicity studies in rats, supported by an upper airway irritation test in mice and corrosive properties of MIT. However, the available data indicate that the mechanism of toxicity is corrosivity. Therefore MIT shall be

labelled as **EUH071** (Corrosive to the respiratory tract), since corrosivity is already covered by the classification Skin Corr 1B.

4.3.3 Conclusions on classification and labelling

In accordance with the provisons of CLP Regulation (EC) No 1272/2008 MIT shall be labelled as **EUH071**(Corrosive to the respiratory tract) while H355 shall be omitted.

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

Summary of the Dossier Submitter's proposal

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

RAC note: The upper airway irritation test is a measure of sensory irritation and whilst it can be used for setting up workplace exposure limits, it is not used for classification purposes.

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

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

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

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

4.4 Irritation

4.4.1 Skin irritation

Skin irritation was determined in rabbits and *in vitro* on human skin construct. Results and conclusion are presented in section 4.5 (Corrosivity) of this report.

4.4.1.1 Non-human information

See section 4.5 (Corrosivity) of this report.

4.4.1.2 Human information

See section 4.5 (Corrosivity) of this report.

4.4.1.3 Summary and discussion of skin irritation

See section 4.5 (Corrosivity) of this report.

4.4.1.4 Comparison with CLP classification criteria

See section 4.5 (Corrosivity) of this report.

4.4.1.5 Conclusions on classification and labelling

See section 4.5 (Corrosivity) of this report.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

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

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

Comments received during public consultation

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

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

The second MSCA also considered that an occupational accident described in the skin sensitisation section is more relevant to the discussion on irritation/corrosion. The DS agreed with the suggestion. The incident was described as follows: "*An accident with MIT was reported when one of the workers in Rohm and Haas was exposed to the substance. In this case blistering and reddening of skin were the signs of exposure. Over the years of manufacturing MIT, no worker has experienced continuing skin problems and none has had to be transferred to other duties due to exposure to chemicals*."

Additional key elements

In the skin irritation study that had 6 animals, rabbits were exposed to MIT for 1 h or 4 hrs under semi-occluded dressing. The DS provided further information about this study, which was used to justify the classification proposal. The following skin findings and skin irritation scores were recorded.

Skin irritation scores after 1 h exposure to MIT:

Parameter	Time after patch removal				
	1 h	24 hrs	48 hrs	72 hrs	7 days
Erythema	3 b	4 c	4 c	4 c,d	4 e
Edema	4 a	4 a	4 a	4 a	0

a – pocketing oedema, b- darkened areas, c-blackened areas, d- blanching, e- concave eschar

Skin irritation scores after 4 hrs exposure to MIT:

Parameter	Time after patch removal					
	1 h	24 h	48 h	72 h	7 days	14 days
Erythema	4 b	4 b	4 b,c	4 b,c	4 b,c	4 d
Edema	4 a	4 a	4 a	4 a	4 a	0

a – pocketing oedema, b- blackened areas, c- blanching, d- concave eschar

The DS did not indicate the irritation scores for individual animals or how many animals the skin effects were observed in. In addition, the DS also tabulated the individual findings from the second skin irritation study, observed after 4 h exposure of rabbits to MIT.

Skin irritation scores in three rabbits up to 14 days after 4 hrs exposure to MIT:

Animal No.	1 hr		24 h		48 h		72 h		7 days		10 days		14 days	
	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed	Ery	Oed
1	1	1	2	1	2	1	2	1	4	*	4	*	4	*
2	3	0	3	0	3	0	3	0	4	*	4	*	4	*
3	1	3	1	2	1	1	1	1	4	*	4	*	4	*

* = not evaluated due to eschar formation

The DS stated that the findings from these 2 studies supported classification of MIT as Skin Corr. 1B; H314. However they did not elaborate on their reasoning.

In addition, in the acute dermal toxicity study, local effects were observed on day one and continued to be observed throughout the study duration (14 days). These effects included blanching, oedema, darkened areas, eschar formation, sloughing, scabbed areas and desiccation.

Assessment and comparison with the classification criteria

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

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

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

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

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

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

At 51.5%, MIT was not corrosive after 3 minutes exposure. However cell viability was reduced to 13.6% following 60 minutes exposure. This result provides supportive evidence for classification of MIT as Skin Corr. 1B or 1C.

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

After 1h and 4h dermal exposure, observations of severe erythema (progressing to score 4 and not reversible) and the blanching of skin with eschar formation are collectively considered to be evidence of corrosivity. RAC agrees with the DS that MIT meets the criteria for classification as Skin Corr. 1B; H314 (Corrosive in >1 of 3 animals following exposure > 3 minutes - ≤ 1 hour, with an observation period of ≤ 14 days).

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

4.4.2 Eye irritation

Eye irritation potential of MIT was not tested since MIT is corrosive to the skin and therefore it is considered to be corrosive also to the eye. In accordance with the Technical Notes for Guidance on data requirements (Chapter 2 Section 6.1.4) MIT was not tested for eye irritation.

4.4.2.1 Non-human information

Not relevant for MIT.

4.4.2.2 Human information

Not relevant for MIT.

4.4.2.3 Summary and discussion of eye irritation

Not relevant for MIT.

4.4.2.4 Comparison with CLP classification criteria

Not relevant for MIT.

4.4.2.5 Conclusions on classification and labelling

Not relevant for MIT.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

No comments were received addressing this endpoint.

Assessment and comparison with the classification criteria

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

4.4.3 Respiratory tract irritation

Respiratory irritation of MIT was tested in upper airway irritation test in mice. The results and conclusions are involved in section 4.3 (STOT SE) of this report.

4.4.3.1 Non-human information

See section 4.3 (STOT SE) of this report.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

See section 4.3 (STOT SE) of this report.

4.4.3.4 Comparison with CLP classification criteria

See section 4.3 (STOT SE) of this report.

4.4.3.5 Conclusions on classification and labelling

See section 4.3 (STOT SE) of this report.

4.5 Corrosivity

Skin irritation was determined in rabbits and *in vitro* on human skin construct. Results are presented in table 10.

Table10: Summary of relevant corrosivities studies

Species	**Test substance**	**Method**	**Average score 24, 48, 72 hrs**		**Reversibility Yes/No**	**Result**	**Reference**
Rabbits New Zealand White	RH-573 Technical 97.8 % active substance	OECD 404	Erythema: 3 min exposure: 1 1 hour exposure: 4 4 hours exposure: 4	Edema: 3 min exposure: 0.4 1 hour exposure: 4 4 hours exposure: 4	3 min exposure: No; edema reversible, **erythema irreversible** 1 hour exposure: No; edema reversible, **erythema irreversible** 4 hours exposure: No; edema reversible, erythema irreversible	**Corrosive**	A6.1.4/01 (Rohm and Haas)
EPIDERM (EPI-200) human epidermal construct	MIT at 1.7 % and 51.5 % in water	OECD 431	na	na	na	1.7 %: not corrosive **51.5%: corrosive (after 60 minutes)**	A6.1.4/02 (Rohm and Haas)
Rabbits New Zealand White	Acticide SR 3267 (49.5 % MIT in water)	OECD 404	Erythema: 2	Edema: 1	7, 10 and 14 days after treatment erythema grade 4 was observed, edema was not evaluated due to eschar formation. Not reversible	**Corrosive**	A 6.1.4-01 (Thor GmbH)

4.5.1 Non-human information

MIT was tested twice for skin irritation **in rabbits**:

In first study (Rohm and Haas) after **3 minutes of exposure** it induced moderate erythema and edema (average erythema score in 6 animals 1.0, average edema score 0.4); edema was no longer present after 7 days, but **erythema persisted for 7 days**. **After 1 and 4 hours of single animal exposure severe erythema and edema were observed (both grade 4), with edema being reversible and erythema irreversible after 7 days and 14 days for 1 and 4 hours exposure, respectively** (Rohm and Haas).

In second study (Thor GmbH) after 4 hours of exposure **erythema and edema of average grade 2 and 1, respectively, were observed after 24, 48 and 72 hours**. Erythema of grade 4 was observed on day 7 and persisted until study termination on day 14. Edema was not evaluated on days 7, 10 and 14 due to eschar formation. **Skin irritating effects of MIT were not reversible during the observation period**

4.5.2 Human information

Skin irritation potential of MIT was studied also in *in vitro* study on human epidermal construct. In this test system MIT was not corrosive at 1.7 % a.s. after 3 and 60 minutes. 51.5 % MIT was also not corrosive after 3 minutes exposure but was corrosive after 60 minutes exposure as indicated by reduction of cell viability to 13.6 % (Rohm and Haas).

Skin irritation potential of MIT was also determined in humans in a 21-day cumulative irritation study. The study is described in Section 4.6.1.2 "Human data" and no cumulative irritation was observed in humans exposed up to and including 0.05 % MIT (equal to 39.5 $\mu g/cm^2$). Cumulative, but not acute, skin irritation in humans was determined at 0.1 % MIT (79 $\mu g/cm^2$) in water (A6.12.6/01, Rohm and Haas).

4.5.3 Summary and discussion of corrosivity

MIT is considered to be corrosive to skin and eyes (eye irritation potential of MIT was not tested since MIT is corrosive to the skin) based on the corrosive effects observed in rabbits exposed to MIT for 3 minutes (6 animals average erythema score 0.1, edema score: 0.4, **erythema persisted for 7 days)**, 1 hour and 4 hours (erythema and edema score 4.0, **erythema irreversible after 7 days)** (Doc IIA, A6.1.4/01) and corrosiveness in human skin epidermal construct **(after 60 minutes exposure to 51.5 % MIT reduction of cell viability to 13.6 %)** (Doc IIA, A6.1.4/02).
Based on the dose selection used in submitted skin irritation/corrosion studies, the SCL for MIT can not be derived. Therefore the generic concentration limit (< 1 % w/w) will apply for the mixtures.

4.5.4 Comparison with CLP classification criteria

CLP:

MIT shall be classified as **Skin corr. 1B, H314** (Causes severe skin burns and eye damage) based on the corrosive effects observed in rabbits exposed to MIT for 3 minutes, 1 hour and 4 hours (DocIIA, A6.1.4/01) and corrosiveness in human skin epidermal construct (DocIIA, A6.1.4/02).

4.5.5 Conclusions on classification and labelling

In accordance with the provisons of CLP Regulation (EC) No 12272/2008 MIT is corrosive to the skin and shall be assigned with pictogram **GHS05,** with signal word "Danger" and with the following hazard statement **H314** (Causes severe skin burns and eye damage).

4.6 Sensitisation

4.6.1 Skin sensitisation

Skin sensitisation of MIT was extensively tested in skin sensitisation assay with method of Buehler and Magnusson-Klingmann and in open epicutaneous method. In addition it was tested in local lymph node assay in mice. Results are summarised in the Table 12a. Additionally, several human skin sensitisation studies were performed and are reported in Table 11b.

4.6.1.1 Non-human information

Skin sensitisation potential has been tested in several animal studies. Results sre summarised in the Table 11a.

Table 11a: Summary table of relevant skin sensitisation studies

Species/ Tested material	Method	Number of animals sensitized/total number of animals	Result	Reference
Guinea pig /Hartley, RH-24,573. (purity, 99.8% a.i.)	OECD 406, Skin sensitisation, Buehler GLP	Induction at 1000, 5000, 15,000 or 30,000 ppm MIT, equivalent to 0.1, 0.5, 1.5 and 3 % MIT Incidence of erythema after challenge with 1000 ppm MIT was 0/10, 0/10, 1/10, and 0/10, respectively. Incidence of erythema after challenge with 5000 ppm a.i. MIT was 0/10, 2/10, 1/10, and 2/10, respectively. Incidence of erythema after challenge at 15,000 ppm a.i. MIT was 1/10, 6/10, 3/10 and 5/10, respectively.	Sensitiser at concentrations greater than ≥0.1 % MIT [or ≥100 µg MIT/cm^2]	A6.1.5/01 (Rohm and Haas)
Guinea pig /Hartley (purity 99.7% a.s.)	OECD 406, Skin sensitisation, Magnusson-Kligman GLP	Induction at 550 or 800 ppm (0.055 or 0.08 %) MIT Challenge of 500 ppm (0.05 %) MIT or 800 ppm (0.08 %) MIT at 24 or 48h, no dermal reactions. Rechallenge phase: 4/20 animals induced at 550 ppm (0.055 %) a.i. exhibited a dermal reaction to the rechallenge application of 1000 ppm a.i. (0.1 %) 5/19 animals induced at 0.08 % a.i. responded to 0.1 % a.i.	Not a sensitiser at concentrations ≤ 0.08 % a.i. [or ≤ 35 µg a.i./cm^2].	A6.1.5/02 (Rohm and Haas)
Guinea pig /Dunkin-Hartley, Acticide SR 3267, (purity 49 % a.i. in water)	OECD 406, Skin sensitisation, Magnusson-Klingmann GLP	First induction: 0.1 % intradermally. Second induction: 10 % topical application under occlusion for 48 hours. Challenge: 1 % topical application under occlusion (24 hours). Positive reaction was observed in 10/10 treated animals in 4/10 intensive	Sensitiser at 1 % concentration of MIT.	A 6.1.5-01 (Thor GmbH)

		erythema and swelling. In control animals no positive reaction was observed.		
Guinea pig /Hsd Poc:DH (SPF), 19.7 % MIT in water.	Skin sensitisation, Open Epicutaneous Method	See table (A6.1.5/03)	Not a sensitiser at concentrations ≤ 0.3 % a.i. [or ≤ 38 µg a.i./cm^2].	A6.1.5/03 (Rohm and Haas)
Mice/ CBA/J; 10.37 % MIT in water.	OECD 429, Local lymph node GLP	Stimulation index was: 2.08 at 0.15 % 2.40 at 0.45 % 2.23 at 0.76 % 6.64 at 1.35 % 4.73 at 1.57 % 6.62 at 1.8 %	Sensitiser at concentrations greater than 0.76 % a.i. (or > 152 µg a.i./cm^2)	A6.1.5/04 (Rohm and Haas)
Mice/ CBA/J; NMMA (99.9%)	OECD 429, Local lymph node GLP	Stimulation index was: 0.81 at 3 % 0.66 at 10 % 0.60 at 30 %	Not sensitiser at concentration up to and including 30 % a.i. [or 6000 µg a.i./cm^2]	A6.1.5/05 (Rohm and Haas)

A skin sensitisation assay according to the Buehler method was performed with MIT. MIT gave positive results at concentrations higher than 0.1 % [or ≥100 µg MIT/cm^2], however insufficient number of animals was used in this test. By Magnusson-Klingmann method 15 % of animals responded to MIT at concentration 0.055 % [or 24 µg a.i./cm^2] and 26 % to MIT_at 0.08 % [or 35 µg a.i./cm^2]. In open epicutaneous test MIT did not induce skin sensitisation at concentrations up to and including 0.3 % [38 µg a.i./cm^2 **]. In local lymph node assay concentrations of MIT greater than 0.76 % [152 µg MIT/ cm^2] gave positive results (DocIIA, A6.1.5/04, Rohm and Haas).**

Skin sensitisation potential of MIT was tested in **another study in guinea pigs according to the Magnusson-Klingmann method.** In animals challenged with 1 % MIT (after 0.1 % intradermal application on day 0 and dermal application of 10 % MIT for 48 hours on days 7 and 8) erythema of various grades were observed in all animals. **In 10/10 animals skin reactions were observed, in 4/10 animals intensive erythema and swelling (DocIIA, A6.1.5-01, Thor GmbH).**

In the open literature several skin sensitisation studies with MIT were published. In a Guinea pig skin sensitisation test MIT was reported to be a weak sensitizer (Bruze et al, 1987), but a strong one (EC = 0.4 % MIT in acetone:olive oil) in mouse local lymph node

assay (Basketter et al., 2003A major metabolite of MIT NMMA was also tested for skin sensitisation potential and gave negative results at concentrations up to and including 30 % [or 6000 µg a.i./cm²] (Rohm and Haas).

4.6.1.2 Human information

Clinical trials of irritation and sensitisation were performed in humans.

Skin sensitisation studies were also performed in humans by repeated repeated insult patch test. Volunteers were exposed to 100, 200, 300, 400, 500 and 600 ppm of MIT (tested substance was 50 % MIT in propylene glycol) for 9 consecutive days, followed by 10-15 days of rest. Thereafter the challenge was performed with the same concentration as used in the induction phase. Results of skin sensitisation studies in humans are reported in Table 11b and show that MIT was a skin sensitizer in 1/116 and 1/210 volunteers exposed to 400 and 500 ppm (0.04 % or 0.05 %), respectively. At lower concentrations (0.01, 0.02, 0.03%) and at 0.06 % MIT did not induce skin sensitisation after 9 consecutive applications.

Table 11b : Summary of skin sensitisation studies in humans

DOSE	SKIN SENSITISATION (POSITIVE/ALL VOLUTEERS)	REFERENCE
0.01 % (3.75 µg/cm^2)	1/98 (1 volunteer was pre-sensitized)	Shelanski, m.V. (2000) (A6.12.6/02 ,Rohm and Haas)
0.02 % (10 µg/cm^2)	0/100	Georgeian K. (2000a) (A6.12.6/03,Rohm and Haas)
0.03 % (15 µg/cm^2)	0/98	Georgeian K. (2000b) (A6.12.6/04 ,Rohm and Haas)
0.04 % (20 µg/cm^2)	1/116	Georgeian K. (2001a) (A6.12.6/05 ,Rohm and Haas)
0.05 % (25 µg/cm^2)	1/210	Georgeian K. (2001b) (A6.12.6/06 ,Rohm and Haas)
0.06 % (30 µg/cm^2)	0/214	Georgeian K. and Vendetti, N. (2002) (A6.12.6/07 , Rohm and Haas)

In another human 21-day cumulative skin irritation/sensitisation study skin sensitisation was determined at 0.1 % MIT in 2/16 volunteers. People were exposed for 24 hours to 1000, 500, 250, 100 and 50 ppm (equivalent to 0.1, 0.05, 0.025, 0.01 and 0.005 %) MIT on 19 mm Hill Top chambers, that equals 79, 39.5, 19.8, 7.9 and 3.9 µg a.s./cm^2, respectively. MIT did not induce cumulative irritation at doses up to and including 500 ppm (39.5 µg a.s./cm^2). **At 0.1 % cumulative skin irritation was observed in one person from day 17 on. Skin sensitisation was observed in 2 people induced and**

challenged with 0.1 % and 1 person induced with 0.1% and challenged with 0.025 and 0.05 % (Doc IIA, A6.12.6/01, Rohm and Haas, Thor GmbH).

An accident with MIT was reported when one of the workers in Rohm and Haas was exposed to the substance. In this case blistening and reddening of skin were the signs of exposure. Over the years of manufacturing MIT no worker has experienced continuing skin problems and none has had to be transferred to other duties due to exposure to chemicals.

Several studies and case reports have been published indicating skin sensitising potential of MIT in humans. Dermatitis patients in several European countries respodened positively to MIT in patch tests. Some studies have shown cross-reactivity of MIT to CMIT/MIT and vice versa. Possible sources of MIT exposure are cosmetics, occupational sources (paints, lacqures, metal working fluids,..) and household products. Some publications are summarised in the following Table 12.

Table 12: Summary table of publications

Study type	**Subject and dose tested**	**Positive response to MIT**	**Reference**
Human patch test, Dept. Of Dermato-Allergology, Gentofte Hospital, Denmark 2010-2012	Patients with contact dermatitis, 2766 Dose: 2000 ppm or 0.2 % MIT	-2010: 2.0 % -2011: 3.0 % -2012: 3.7 %	Lundov et al., 2013, Contact Dermatitis, 69(5):271-275
Human patch study, 2009-2012, Information Network of Departments of Dermatology, data from Germany, Austria and Switzerand	28,922 dermatitis patients Dose: 500 ppm or 0.05 % MIT	Average 3.83 % ; 1.94 % positive in 2009, 6.02 % in 2012	Utter et al., 2013, Contact Dermatitis, 69, 231-238
Human patch test, Finland, 2006-2008	10,821 dermatits patients Dose: 1000 ppm or 0.1 % and 300 ppm or 0.03 % MIT	1.4 % positive at 1000 ppm (0.1 %) and 0.6 % at 300 ppm (0.03 %) MIT	Ackermann et al., 2011, Contact Dermatitis, 64 (1), 49-53
Human patch test, Sweden, May 2006-February 2010	2,536 dermatitis patients Dose: 2000 ppm or 0.2 % MIT	1.5 % on average were positive in 5 years (annual prevalence 1.1-2.2 %) 30 % of MIT-sensitized individuals were occupationally exposed to MIT, 45 % (5/11) of them	Lundov et al., Contact Dermatitis, 2010, 63, 164-167

<table>
<tr><td></td><td></td><td>were painters</td><td></td></tr>
<tr><td>Human patch study, 2009-2012
Leeds Center for Dermatology, UK</td><td>Patients with contact dermatitis
-2009: 349; 0.02 %MIT
- 2010:771; 0.02 % MIT
-2011:611; 0.02 % MIT and 238; 0.2 % MIT
- 2012 (Jan-Jun):325; 0.02 and 0.2 % MIT</td><td>- 2009: 0.6% (0.02 % MIT)
-2010: 1.1 % (0.02 % MIT)
-2011:
1.8 % (0.02 % MIT),
3.8 % (0.2 % MIT)
-2012:
2.5 % (0.02 % MIT),
4.6 % (0.2 % MIT)</td><td>Urwin and Wilkinson, 2013, Contact Dermatitis, 68, 250-256</td></tr>
<tr><td>Analysis of human patch tests, Denmark</td><td>36,147 patients with contact dermatitis, 219 painters,

41 painters tested with MIT

Dose: not reported</td><td>11/41 painters (27%) positive for MIT</td><td>Mose et al., 2012, Contact Dermatitis, 67 (5)293-297</td></tr>
<tr><td>Repeated open application test (ROAT) and patch test were performed</td><td>11 patients sensitised to MIT

Patch test: 12 concentrations: 0.2, 0.1, 0.05, 0.03, 0.015, 0.01, 0.005, 0.0015, 0.0007, 0.0005, 0.00035, 0.000035% MIT, twice daily.

ROAT: 0.0007, 0.00035, 0.000035% MIT.

The use of cream protected with MIT was mimiced</td><td>Endpoint: Elicitation

Patch test:<table><tr><td>Dose (%)</td><td>Reaction</td></tr><tr><td>0.2</td><td>10/11</td></tr><tr><td>0.1</td><td>10/11</td></tr><tr><td>0.05</td><td>10/11</td></tr><tr><td>0.03</td><td>10/11</td></tr><tr><td>0.015</td><td>8/11</td></tr><tr><td>0.01</td><td>7/11</td></tr><tr><td>0.005</td><td>6/11</td></tr><tr><td>0.0015</td><td>0</td></tr><tr><td>0.0007</td><td>0</td></tr><tr><td>0.00035</td><td>0</td></tr><tr><td>0.00035</td><td>0</td></tr></table>
ROAT:<table><tr><td>Dose (ppm)</td><td>Reaction</td></tr><tr><td>0.0007</td><td>7/11</td></tr><tr><td>0.00035</td><td>7/11</td></tr><tr><td>0.000035</td><td>2/11</td></tr></table></td><td>Lundov et al., 2011, Contact Dermatitis, 64, 330–336</td></tr>
</table>

4.6.1.3 Summary and discussion of skin sensitisation

MIT has been shown to be a skin sensitizer in local lymph node assay, Buehler test, Magnusson-Klignamm skin sensitisation assay and in open epicutaneous test and should be classified **Skin sensitiser 1A, H317** (May cause an allergic skin reaction)**.**

MIT has also been tested for skin sensitisation in humans. MIT (ca. 50 % in propylene glycol) was a skin sensitizer in 1/116 and 1/210 volunteers exposed to 400 ppm (0.04 % or

20 µg/cm^2) or 500 ppm (0.05 % or 25 µg/cm^2). At lower concentrations (0.01 % or 3.75 µg/cm^2, 0.02 % or 10 µg/cm^2, 0.03 % or 15 µg/cm^2) and at 0.06 % (or 30 µg/cm^2) MIT did not induce skin sensitisation after 9 consecutive applications followed by 10-15 days rest before challenge. The study is designed to maximise exposure to the test substance to try to generate a response, the exposure is repeated nine times over a 21 days period and involves occlusion and can be considered an extreme exposure scenario. In addition, the study uses a formulated product diluted in water which may affect the sensitisation potential due to vehicle effects. Given the lack of dose-response in this study, it's suitability for defining an SCL is questionable.

Additionally, in 2013 MIT was a subject of evaluation of the Scientific Committee on Consumer Safety regarding the current concentrations of MIT in cosmetic products (*Scientific Committee on Consumer Safety (SCCS) opinion on Methylisothiazolinone (P94), Submission II (Sensitisation only). SCCS/1521/13 – 12 December 2013 - revision of 27 March 2014*"). The conclusions of the SCCS opinion are cited below:

"*1. On the basis of the new evidence in relation to sensitising potential, does the SCCS consider Methylisothiazolinone (MI) still safe for consumers, when used as a preservative in cosmetic products up to concentration limit of 100 ppm? If no, it is asked for the SCCS to revise this concentration limit on the basis of information provided.* Current clinical data indicate that 100 ppm MI in cosmetic products is not safe for the consumer. For leave-on cosmetic products (including 'wet wipes'), no afe concentrations of MI for induction of contact allergy or elicitation have been adequately demonstrated. For rinse-off cosmetic products, a concentration of 15 ppm (0.0015%) MI is considered safe for the consumer from the view of induction of contact allergy. However, no information is available on elicitation.
2. *Does the SCCS have any further scientific concerns with regard to the use of Methylisothiazolinone (MI) in cosmetic products?*
MI should not be used as an addition to a cosmetic product already containing MCI/MI. More frequent review of data (than suggested in SCCS/1482/12) to monitor sensitisation frequencies of MI and related isothiazolinone preservatives is recommended. This permits trends in consumers' sensitisation to be observed and timely intervention to be taken. Information on the actual concentration of MI present in individual cosmetic products will allow future evaluation of safe concentrations. Labelling is only helpful to a consumer who has a known (established by diagnostic patch test investigations) allergy. It is unknown what proportion of the general population is now sensitized to MI and has not been confirmed as sensitized. Since MI is widely used in other consumer products (eg. detergents, paints), exposures from such sources should also be assessed. Consumers cannot find information on the presence of MI in products except in cosmetics and household detergents because, as yet, there is no harmonised classification of MI as a skin sensitizer. The risk for skin sensitisation by MI is at least equivalent to that of other substances which have received a harmonised classification according to the CLP Regulation."

It has to be stressed that cosmetic products are intentionally applied to the skin and at higher doses, that is why setting the lower maximum concentration seems reasonable for cosmetic products.

Skin sensitisation after exposure to MIT has been reported in several European countries in contact dermatitis patients. Some case reports on allergic reactions to MIT have also been published. From a scientific point of view the robustness of these data and their

suitability for classification purposes is questioned, as many of the reports were not peer reviewed, adequate reporting and presentation of data is lacking, and exposure was not sufficiently characterized.

Based on skin sensitisation studies in animals and humans setting specific concentration limits for skin sensitisation 0.06 % seems justified, which is lower than the generic concentration limit for skin sensitizer 1A. However, the proposed SCL may not be protective enough for some MIT pre-sensitized individuals as indicated in published studies.

4.6.1.4 Comparison with CLP classification criteria

sensitisationsensitisationsensitisationsensitisation**CLP:**

MIT shall be classified as Skin sens. 1A with **H317** (May cause an allergic skin reaction.) on the basis of the positive results from animal and human tests:

- local lymph node assay; the stimulation index was above 3 (6.65) at MIT concentration 1.35 %, what fulfills the criteria for Skin sens. 1A, EC3 value ≤ 2 % (DocIIA, A6.1.5/04).

- Magnusson-Klingmann skin sensitisation assay; in 10/10 animals signs of skin sensitisation were observed after induction with 0.1 % MIT, what fulfills the criteria for Skin sens. 1A, where ≥ 30 % should respond positively after induction with concentration ≤ 0.1 % (DocIIA, A6.1.5-01).

- Positive response in human repeated insult patch study with methylisothiazolone at concentration of 0.04 and 0.05 % in propylene glycol, with 1/116 and 1/210 being positive, respectively, is suportive evidence of skin sensitisation in humans. However due to lack of dose response in the respective study, these results have not been considered for the proposal of classification.

- Diagnostic patch tests showed that there is relatively high and substancial evidence of allergic contact dermatitis in relation to relatively low exposure. Relatively high frequency of occurance of skin sensitisation for MIT was demonstrated in dermatitis patients who reacted positively to MIT in more than 1 % in patch tests and in several collated clinics data indicating ≥ 1 % response. Exposure of individuals that responded positively to MIT is at relatively low dose (< 0.1 %), but frequency of exposure was relatively high ≥ once/day and ≥ 100 exposures per year (either from occupational, household, cosmetic or other exposure). These results published in the open literature further support the sub-classification of MIT in category skin sensitizer 1A.

4.6.1.5 Conclusions on classification and labelling

sensitisation**In accordance with the provisons of CLP Regulation (EC) No 1272/2008** MIT shall be as **Skin sens.1A , H317** (May cause an allergic skin reaction)

According to the criteria of Guidance on the Application of the CLP criteria, MIT is considered to be a strong sensitizer.

In addition, based on skin sensitisation studies in animals and humans setting lower specific concentration limits for skin sensitisation of 0.06 % seems justified. **Thereafter**

the special labeling requirement is applied for mixtures not being classified for skin sensitization, but containing more than 0.006 % of MIT; EUH 208 – Contains 2-**methylisothiazol-3(2H)-one. May produce an allergic reaction.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Dossier Submitter proposal submitted in the CLH report

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

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

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

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

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

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

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

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

MIT.

Revised position of the Dossier Submitter following the Public Consultation

After the public consultation, the DS confirmed that MIT should be classified Skin Sens 1A, H317, but that the data and weight of evidence analyses submitted during the public consultation show that the proposed SCL of 0.06% (600 ppm) may not be sufficiently protective for sensitised individuals. Therefore, the DS responded that a lower limit should be defined. The DS emphasised that all mixtures containing MIT should be automatically labelled with EUH208: "Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction."

Comments received during public consultation

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

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

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

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

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

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

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

A third manufacturer commented that the available animal studies, including the LLNA, GPMT and Buehler tests, showed MIT to be a potent sensitiser. In their opinion the human prevalence and clinical data also pointed to MIT being a potent sensitiser, but these data were not sufficiently reliable for setting an SCL since the exposures that had

caused induction in the affected individuals had not been defined. Similarly, repeated open application test (ROAT) data from already sensitised patients were not suitable, as they related only to challenge, not induction concentrations. The only relevant human data came from the HRIPT, where exposure was carefully controlled. However, the study with MIT was not considered sufficiently robust for regulatory purposes, and the third manufacturer preferred that the GCL of 0.1% for a potent sensitiser be applied to MIT.

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

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

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

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

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

Responding to these comments, the DS acknowledged that the proposed SCL of 600 ppm was not well justified. They also concluded that 0.1% was not sufficiently protective, acknowledging the studies reporting an increasing incidence of confirmed MIT sensitised

individuals, skin sensitising reactions to MIT at concentrations below 600 ppm and wide use of MIT in industrial and consumer products.

In their comments; the SCCS agreed that an SCL is needed, citing both the results of the HRIPT with MIT, the unusually high number of sensitisation cases to MIT, and reported increase in numbers up to 6-fold among consumers and workers patch tested for MIT sensitisation in different areas of Europe. Although it had ethical concerns about the HRIPT in general, the results of the HRIPT with MIT appeared to show induction responses to levels as low as 100 ppm. Although a clear dose-response relationship was not observed, there were no scientific reasons to specifically disregard the results from the lower dose groups. The results could not be explained by the irritant nature of MIT and were more likely a consequence of the challenge dose not being sufficiently high.

The SCCS also stated that:

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

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

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

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

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

However, other comments sought a much lower limit for labelling with EUH208. In some cases, including from the SCCS and one member state, no limit at all was recommended. It was suggested that products containing MIT should be labelled with the name of the sensitising substance, but with no lower concentration limit because they considered that the criteria in CLP Annex II to label sensitisers with EUH208 down to 1/10 of the GCL or

SCL is not sufficiently protective to prevent elicitation of allergic contact dermatitis in those already sensitised to MIT.

The Swedish Institute of Environmental Medicine and a statement issued on behalf of over 140 experts from dermatology, allergenicity, epidemiology, occupational medicine and health education across the EU, also commented that no limit should be set for the application of EUH208 to MIT.

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

Additional key elements

(a) *Skin sensitisation studies in animals*
In response to specific comments from the SCCS about the animal studies summarised in the CLH report, the DS provided the following revised tabulated summary of the data.

Species/ Tested material	Method	Number of animals sensitised/total number of animals	Result	Reference
Guinea pig /Hartley, (purity, 99.8% a.i.)	OECD 406, Buehler GLP	Induction at 1000, 5000, 15,000 or 30,000 ppm MIT, equivalent to 0.1, 0.5, 1.5 and 3 % MIT Incidence of erythema after challenge with 1000 ppm MIT was 0/10, 0/10, 1/10, and 0/10, respectively. Incidence of erythema after challenge with 5000 ppm a.i. MIT was 0/10, 2/10, 1/10, and 2/10, respectively. Incidence of erythema after challenge at 15,000 ppm a.i. MIT was 1/10, 6/10, 3/10 and 5/10, respectively.	Sensitiser	A6.1.5/01 (Rohm and Haas)
Guinea pig /Hartley (purity 99.7% a.s.)	**OECD 406, Magnusson-Kligman** **GLP**	Induction at 550 or 800 ppm (0.055 or 0.08 %) MIT Challenge of 500 ppm (0.05 %) MIT or 800 ppm (0.08 %) MIT at 24 or 48h, no dermal reactions. Rechallenge phase: 4/20 animals induced at 550 ppm (0.055 %) a.i. exhibited a dermal reaction to the rechallenge application of	Not a sensitiser.	A6.1.5/02 (Rohm and Haas)

		1000 ppm a.i. (0.1 %) 5/19 animals induced at 0.08 % a.i. responded to 0.1 % a.i.		
Guinea pig /Dunkin-Hartley, Acticide SR 3267, (purity 49 % a.i. in water)	OECD 406, Skin sensitisation, Magnusson-Klingmann GLP	First induction: 0.1 % intradermally. Second induction: 10 % topical application under occlusion for 48 hours. Challenge: 1 % topical application under occlusion (24 hours). Positive reaction was observed in 10/10 treated animals in 4/10 intensive erythema and swelling. In control animals no positive reaction was observed.	Sensitiser	A 6.1.5-01 (Thor)
Guinea pig /Hsd Poc:DH (SPF), 19.7 % MIT in water.	Skin sensitisation, Open Epicutaneous Method	For induction and challenge the same concentration of MIT in ethanol/aqua bidest. (40 % eth.) was applied. Doses used/positive skin reaction: -18 % 4/8 -1.5% 1/8 -0.6% 1/8 -0.4% 3/8 -0.25% 1/8 -0.15% 0/8 - control 1/8	Sensitiser	A6.1.5/03 (Rohm and Haas)
Mice/ CBA/J; 10.37 % MIT in water.	OECD 429, Local lymph node GLP	Stimulation index was: 2.08 at 0.15 % 2.40 at 0.45 % 2.23 at 0.76 % 6.64 at 1.35 % 4.73 at 1.57 % 6.62 at 1.8 % EC3=0.86 %	Sensitiser	A6.1.5/04 (Rohm and Haas)

(b) *Human repeated open application patch tests*
In response to the public consultation, the DS provided this updated information.

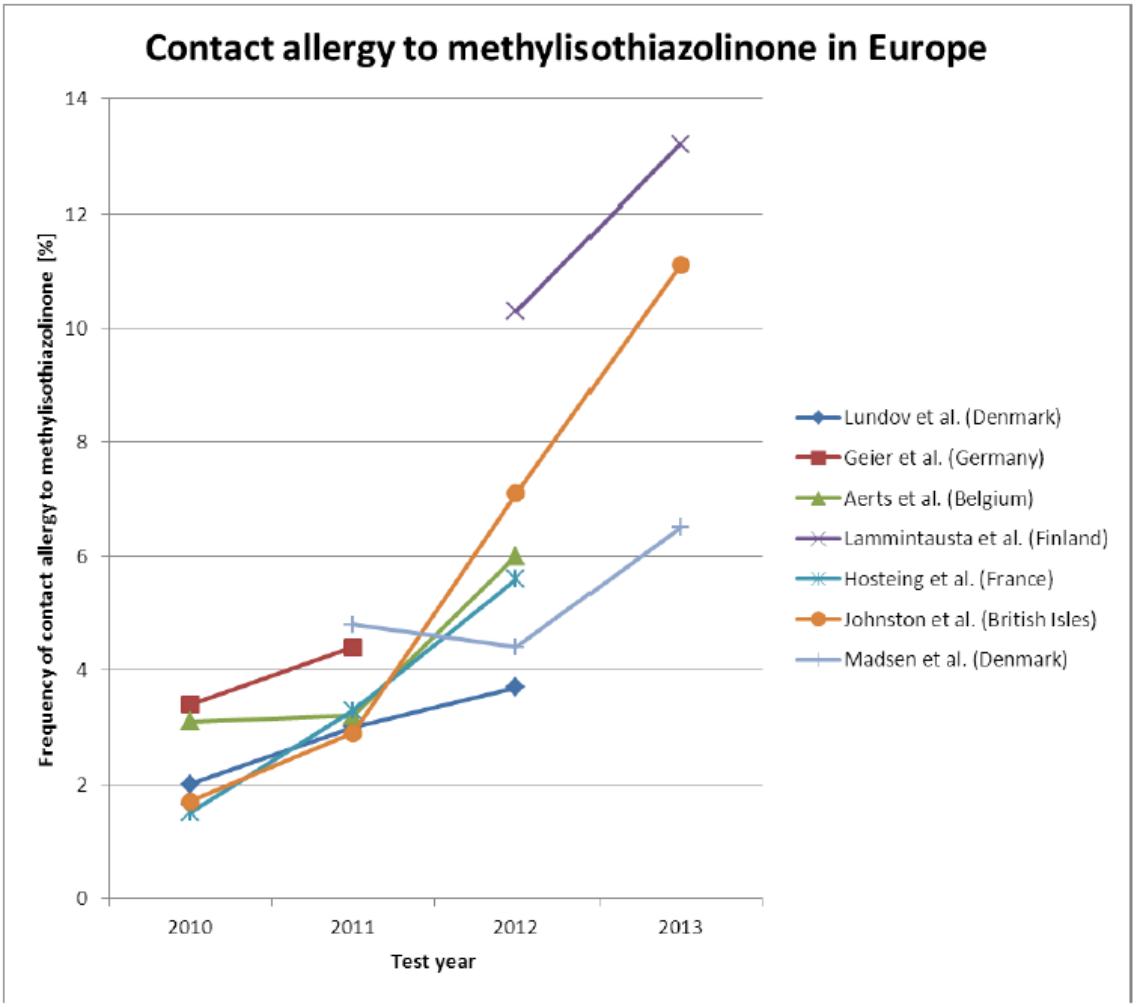
<table>
<tr><th>Study type</th><th>Subject and dose tested</th><th>Positive response to MIT</th><th>Reference</th></tr>
<tr><td>Repeated open application test (ROAT)</td><td>19 MIT positive individuals
ROAT: 50 and 100 ppm MIT applied in liquid hand soap, 5×/day, until positive reaction was observed or day 21.</td><td>Endpoint: Elicitation
ROAT:<table><tr><td>Dose (ppm)</td><td>Reaction</td></tr><tr><td>100</td><td>10/10</td></tr><tr><td>50</td><td>7/9</td></tr></table></td><td>Yazar et al., 2015, British Journal of Dermatology, 173:115-122.</td></tr>
<tr><td>Repeated open application test (ROAT) and patch test were performed</td><td>11 patients sensitised to MIT
Patch test: 12 concentrations: 0.2, 0.1, 0.05, 0.03, 0.015, 0.01, 0.005, 0.0015, 0.0007, 0.0005, 0.00035, 0.000035% MIT, twice daily.
ROAT: 0.0007, 0.00035, 0.000035% MIT.

The use of cream protected with MIT was mimicked</td><td>Endpoint: Elicitation
Patch test:<table><tr><td>Dose (%)</td><td>Reaction</td></tr><tr><td>0.2</td><td>10/11</td></tr><tr><td>0.1</td><td>10/11</td></tr><tr><td>0.05</td><td>10/11</td></tr><tr><td>0.03</td><td>10/11</td></tr><tr><td>0.015</td><td>8/11</td></tr><tr><td>0.01</td><td>7/11</td></tr><tr><td>0.005</td><td>6/11</td></tr><tr><td>0.0015</td><td>0</td></tr><tr><td>0.0007</td><td>0</td></tr><tr><td>0.00035</td><td>0</td></tr><tr><td>0.00035</td><td>0</td></tr></table>ROAT:<table><tr><td>Dose (ppm)</td><td>Reaction</td></tr><tr><td>100</td><td>7/11</td></tr><tr><td>50</td><td>7/11</td></tr><tr><td>5</td><td>2/11</td></tr></table></td><td>Lundov et al., 2011, Contact Dermatitis, 64, 330–336</td></tr>
</table>

(c) *Allergic contact dermatitis due to MIT (summary with references provided by the SCCS during the public consultation)*

The first cases reported of MIT sensitisation published in 2004 and 2006 concerned workers. Later cases from consumer exposure to cosmetics were published in 2010. This has been followed by an unprecedented rapid increase in contact allergy to MIT as presented in SCCS opinions in 2013 and 2015 concerning MIT (see Figure below). The risk from exposure to cosmetics, stay-on and rinse-off are well recognized and described in the two opinions. A considerable proportion of cases are due to occupational exposures. Several occupations with an increased risk have been identified such as painters, beauticians and machine operators, mechanics, health care workers, hairdressers, café and restaurant workers and manufacturing workers. Allergic reactions have in particular been related to detergents, metal working fluids, soluble oils, glues, paints, lacquers, concentrates, soaps for hand wash, industrial hand cleansers, barrier creams and wet wipes. Workers may develop severe allergic hand eczema, which may cause them to lose their job.

MIT has been shown to evaporate from fresh paint for at least 42 days. Persons sensitised to MIT may react to the airborne exposures to MIT at the work place, at home or in public by severe allergic contact dermatitis at the exposed areas. Cases with various respiratory symptoms to MIT have also been published.



(d) Use concentrations of MIT (summary with references provided by the SCCS during the public consultation)

Painters have a high risk of sensitisation to MIT, and cases due to paint exposure among consumers have also been reported. In a European study wall paints (n=71) were randomly purchased in retail outlets in five European countries. MIT was identified in 93.0% n=66 of the purchased paints. The MIT concentration ranged from 0.7 to 180.9 ppm and the majority of products contained MIT at 100 ppm (0.01%) or lower.

A search in the Danish Product Register Database, in which the composition of primarily hazardous chemical products for occupational use are registered, showed that 884 products were registered to contain MIT. The top three product types containing MIT were paint and varnishes, cleaning/washing agents and polishing agents. The mean concentrations ranged from 3 ppm (rinsing agents for dish washing machines) to 1.1% (11000 ppm in concentrates/biocides). Among 31 product categories, 23 (74%) had average concentrations of MIT below 100 ppm (0.01%) and 19 (61%) product categories had maximum concentrations of MIT below 300 ppm (0.03%) (Friis et al. 2014). MIT in up to 100 ppm in cosmetic products has caused many consumers to become sensitised.

(e) A further LLNA study, conducted in 2002, was submitted by industry at the RAC 36 plenary meeting. In this additional study, a water based formulation of MIT (MIT content = 50.50%) was administered to female mice at concentrations of 0%, 2.5%, 5% and 10% (w/v) in ethanol:water, 1:1 (v/v) on 3 consecutive days. All treated animals survived the scheduled study period. No test item-related clinical signs were observed.

The results obtained from this study showed a clear dose-response relationship and are tabulated below.

Concentration of the water-based formulation % (w/v)	S.I.
2.5	1.9
5	6.5
10	16.0

Since the SI ≥ 3 at both 5% and 10% concentrations of the test material the study shows that this water-based formulation of MIT is a sensitiser at concentrations ≥5%. From these results, it can be established that 2.5% < EC3 < 5% in this test. As the formulation contained approx. 50% MIT, this equates to 1.25% < EC3 < 2.5% for MIT itself. On this basis, the potency of MIT in this test would be regarded as moderate to strong (the guidance value for strong potency is an EC3 ≤ 2%).

Assessment and comparison with the classification criteria

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

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

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

		≤50% response at 3% topical induction concentrations of MIT (increasing response rates were seen as the challenge concentration was increased from 0.1, 0.5 and 1.5% MIT).	
Local lymph node assay (Rohm & Haas study)	EC3 value ≤2%	EC3 value at 0.86%	The EC value meets the criteria for cat. 1A

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

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

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

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

Specific concentration limit and additional labelling with phrase EUH208.

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

The Guidance on the Application of CLP Criteria (Version 4.1 – June 2015) describes how SCLs for skin sensitisation can be set based on animal test data. The tests should be on the substance itself, not on mixtures. Substances classified as Skin Sens. 1A may be further defined as being of extreme or strong potency. The recommended SCLs for these two different classes of potent sensitiser are 0.001% and 0.1%, respectively. However, the guidance also indicates that another value could be applied if supported by reliable data. Such data could be human data for which the exposures leading to sensitisation are defined.

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

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

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

The relevant animal data are summarised below.

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

		potency
Local lymph node (Rohm & Haas study)	EC3 value ≤0.2%	EC3 value at 0.86% **Not indicative of extreme potency**
Local lymph node (Thor)	EC3 value ≤ 0.2%	1.25% < EC3 < 2.5% **Not indicative of extreme potency**

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

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

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

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

In RAC's opinion, sufficient information is available to conclude that MIT has extreme

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

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

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

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

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

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

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

4.6.2 Respiratory sensitisation

Currently no respiratory sensitisation study is required. Respiratory sensitisation of MIT was not tested and no classification in regard to respiratory sensitisation is proposed for MIT.

4.6.2.1 Non-human information

No data available

4.6.2.2 Human information

So far several cases of airborne allergic contact dermatitis and systemic contact dermatitis were observed assumed to result from the airborne exposure to MIT from recently painted walls (Lundov et al., 2011, Kaae et al, 2012, Alwan et al, 2014). Just some of reported cases are presented in the following table.

Table 13a: Some of reported cases

Study type	Subject and source of MIT exposure	Reference
Case report – 2 cases	Casino worker: contact dermatitis, paint preserved by MIT, patch test positive with 0.2% MIT. MIT/CMIT in soap, but hands not affected. Moved to renovated flat: headache, dermatits on abdomen. Moved out; OK. Paints containing MIT. Participant in dose-response MIT study. Painted walls at home with paint containing BIT, CMIT/MIT and MIT (CMIT/MIT low concentration). Facial erythema, cought, difficulty breathing, hospitalization. Returned home; dermatitis reoccured at sites exposed during patch test 2 months earlier.	Lundov et al., 2011, Contact Dermatitits, 65, 175-85.
Case report- single case	A 23-years old non-atopic woman with facial dermatitis. Onset after 2 months of working in the freshly painted reataurant. She tested positive in patch test with MIT/CMIT (0.01 % in water), 0.2 % MIT in water and some metals (nickel, palladium, cobalt). Allergy was assumed to be induced by MIT in paint and elicited by MIT in a cosmetic product. When she stopped using the cosmetic product symptoms were rapidly cleared.	Kaae at al, 2012, Contact Dermatitis, 66, 341-2.
Case report – single case	A 3-years old girl was treated for 10 weeks lasting perioral dermatitis. She tested positive in patch test to MIT/CMIT, MIT and some cream. As an infant the girl suffred from atopic dermatitisin diaper area. Her mother used wet wipes containing MIT than; at onset of dermatitis family also used a fabric softener, hair conditioner and shampoo	Alwan et al, 2014. Contact Dermatitis,70, 320-1.

	containing MI.The perioral dermatitits occurred after moving to a newly painted apartment. After 5 months the family moved to another newly painted apartment and the dermatitis reoccurred again.	

The information does not allow the conclusion on respiratory sensitisation following MIT exposure.

4.6.2.3 Summary and discussion of respiratory sensitisation

Four cases of dermatitis were observed after inhalation exposure to MIT from the wall paint. However, reported cases of airborne contact dermatitis were not confirmed by patch testing with the paints. **Though, the information doesn't allow the conclusion on respiratory sensitisation potential of MIT.**

4.6.2.4 Comparison with CLP classification criteria

- Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory sensitisers. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in Table 3.4.1 of CLP and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.
  Regarding animal data, no formally recognised and validated animal tests currently exist for respiratory sensitisation. However, data from some animal studies may be indicative of the potential of a substance to cause respiratory sensitisation in humans (CLP Annex I, 3.4.2.1.3) and may provide supportive evidence in case human evidence is available. No studies are available in guinea pigs and no specific investigations on Immunoglobulin E have been conducted in mice.

  Regarding human data, substances shall be classified as respiratory sensitisers if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity. This is further described in the CLP Annex I, 3.4.2.1.2.

  In view of the above, it can be concluded that in the absence of available studies in animals, specific investigations and reported cases of hypersensitivity in humans, MIT does not fulfil the criteria for respiratory sensitisation.

4.6.2.5 Conclusions on classification and labelling

Data not sufficient.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

Respiratory sensitisation of MIT was not tested.

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

4.7 Repeated dose toxicity

Table 13b presents a summary of results obtained after repeated dose toxicity administration of MIT, respectively.

Table 13b: Summary table of relevant repeated dose toxicity studies of MIT

Route	Duration of study	Species Strain Sex no/group	Dose levels, frequency of application	Results	LO(A)EL	NO(A)EL	Reference
Oral	28 days	Rats, Wistar, M/F, 5/sex/ group	0, 10, 28.6 and 71.2 mg MIT/kg bw/day; gavage (purity 50.7% a.s.) 14 days recovery group	Animals treated with 71.2 mg MIT/kg bw/day: lethargy during week 3 and 4, 1 male and 4 females died, slight reduction in the weekly body weight gain and feed consumption during the experiment.	71.21 mg MIT/ kg bw/day	28.59 mg MIT/ kg bw	A6.3-01 (Thor GmbH)
Oral	3 months	Rats, Crl:CD® BR, M/F, 10/sex/	0, 75, 250, 1000 ppm; drinking water available *ad*	Effects on body weight, food and water consumption were noted in males at 1000 ppm (66 mg/kg bw/day). There	66 mg/kg bw/day for males, 94 mg/kg bw/day	19.0 mg MIT/kg bw/day in males and 24.6	A6.4.1.a/01 (Rohm and Haas)

		group	*libitum* (purity 97,5% a.s.)	was no evidence of systemic toxicity or gross and microscopic pathology at doses up to and including 19 and 24.6 mg/kg bw/day for males and females, respectively.	for females (1000 ppm)	mg/kg bw/day in females (250 ppm)	
Oral	3 months	Dogs, Beagle, M/F, 4/sex/ group	0, 100/130, 400, 1500 ppm; daily diet (purity 50% MIT in water)	Both sexes at 1500 ppm had decreased body weight and food consumption due to reduced food intake during first 3 or 4 weeks; later body weight gain was comparable to control.	41 mg/kg bw/day equivalent to 1500 ppm	9.9 mg/kg bw/day for males and 11.1 mg/kg bw/day for females, (400 ppm)	A6.4.1.b/01 (Rohm and Haas and Thor GmbH)
Oral	90 days	Rats, Wistar, M/F, 10/sex/ group	0, 7.52, 15.05 and 30.09 mg MIT/kg bw/day; gavage (purity 50.7% a.s.) 28 days recovery group	Increased spleen weight was observed at 30.09 mg/kg bw/day. Other findings observed in this study were considered incidental and not adverse.	/	30.09 mg MIT/ kg bw/day	A6.4.-01 (Thor GmbH)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Acticide M50 (49 % MIT in water) was administered to rats by gavage for 28 consecutive days at 0, 10, 28.6 and 71.2 mg MIT/kg bw/day according to the OECD Guidance 407. Animals of the control and high-dose groups were also observed during a 14-day recovery period.

Six animals that were exposed to 71.2 mg/kg bw/day died during the treatment (1 male during week 2, 3 females in week 1, 2 and 4). During 3rd and 4th week 4/5 males treated with high dose and 5/5 males and 1/5 females of the high dose recovery group were lethargic. Additionally, slightly reduced weekly body weight gain and feed consumption were observed in this group. Clinical chemistry analysis performed at the end of the treatment period revealed significant, but marginal reduction in sodium values in males in all the dose groups that was not considered to be biologically significant. Reduction in potassium values in mid and high dose group males was also observed, but the values measured were within the normal range, therefore the decrease was considered incidental. At the end of the recovery period, all the clinical chemistry values in high dose recovery

group were comparable to control recovery group. Clinical chemistry analysis of high dose female group revealed decreased AST, increased total bilirubin, increased phosphorus and increase in total protein. All values were within historical control data range.

In males absolute and relative weight of prostate was significantly reduced in low and high dose group, but no histopathological changes were observed in the prostate. In high dose recovery group, absolute weight of testes and epididymides was significantly less ($p<0.05$) as compared to control recovery group, however, relative weight of these organs was comparable to control recovery group; hence, this variation was considered to be incidental. Relative liver weight in males was significantly increased in mid and high dose group in the absence of histopathological changes and was considered to be incidental. In low and mid dose females increased relative weight of kidneys was observed. Observed variation in relative kidney weight was considered to be incidental as relative weight of kidney in high dose group was comparable to control group. In high dose recovery group, relative weights of organs were comparable to control recovery group.

Gross and histopathological findings observed were not considered treatment related and were recorded either both in control and treatment groups at comparable levels or only in a few animals without consistent pattern and were in conformity with historical control data, hence were considered as spontaneous/incidental findings.

In 28-days oral toxicity study in rats LOAEL 71.2 mg MIT/kg bw/day was determined based on lethargy and deaths observed at this dose. NOAEL was 28.6 mg/kg bw/day (Thor GmbH).

The sub-chronic toxicity of MIT by the oral route has been investigated in a 90-day study in rats. Rats received 0, 75, 250 and 1000 ppm MIT in the diet. There were no treatment-related clinical signs, deaths, ophthalmoscopic findings, or changes in haematology and clinical chemistry. Decreases in water consumption were observed in all groups of males and ≥ 250 ppm MIT treated females throughout the entire treatment period. There was a clear dose response in decreased water consumption; however, there were no corresponding changes in the gross pathology or histopathology indicative of treatment-related irritation in the oral cavity, esophagus, or gastrointestinal tract. Decreased water consumption was assigned to palatability of water containing MIT. Decreased body weight and food consumption were most likely associated with decreased water intake. No treatment-related effects on organ weights, gross pathology and histopathological changes were observed. NOEL 19 and 25 mg/kg bw/day (equivalent to 250 ppm) for males and females, respectively, were determined in this study based on decreased body weight and food consumption at 1000 ppm. No systemic toxicity was observed (Rohm and Haas).

In a second sub-chronic oral toxicity study, MIT was administered daily to dogs in the diet for 90 days. Dogs were exposed to 100/130, 400 and 1500 ppm MIT in diet. No treatment-related clinical signs of toxicity, ophthalmoscopic changes, no haematological, clinical chemistry parameters and urinalysis changes were observed. Food consumption was decreased in males and females at 1500 ppm. However, the food intake was reduced only in the first two weeks of the study, probably due to adaptation of animals on food containing MIT. Test article-related decrease in body weight was observed in both sexes at 1500 ppm. From week 3 to study termination weekly body weight gains were comparable in control and 1500 ppm group. No treatment-related effects on organ

weights, gross pathology and histopathological changes were observed. In this study NOEL 9.9 and 11 mg/kg bw/day (equivalent to 400 ppm) was determined for males and females, respectively, based on decreased food consumption and body weight. LOEL was 41 mg/kg bw/day. No systemic toxicity was observed in this study (Rohm and Haas).

In another oral subchronic toxicity study in rats, the animals were administered Acticide 50 M, containing 50.7 % MIT in water, by gavage at 7.52, 15 and 30 mg MIT/kg bw/day for 90 consecutive days.

Symptoms like nasal discharge, diarrhoea, lethargy, rhinorrhoea, piloerection and wryneck were observed sporadically in the experimental animals irrespective of sex and dose. One male treated with 30 mg/kg bw/day was found dead on 54th day of experiment; this death is considered to be incidental. Transient weekly increases in feed consumption were observed in males in high dose recovery group, but did not reach statistical significance. Some variations in clinical chemistry parameters were observed in all dose groups, but all of them were within the range of historical control data.

Changes in sperm parameters were observed in males exposed to MIT. Sperm motility was reduced in the high dose group, but it was within the historical control data range. Dose dependent reduction in the number of testicular sperm heads in testes was observed in animals treated with MIT. Although significantly reduced, the values are within historical control data range. In addition in two-generation study no effect on sperm count was observed after exposure to higher concentrations of MIT. Considering the fact that epididymal sperm count was not reduced, no change in testes weight and no histopathological changes were observed, reduction being within the historical data range, no effect observed in recovery group and in reproduction toxicity study, the effect on testicular sperm number is not biologically relevant. Morphological examination of the sperm samples obtained from cauda epididymis revealed statistically significant increase in per cent of abnormal sperms in all the treatment groups (2.2, 2.55 and 2.67 % in animals treated with 7.5, 15 and 30 mg/kg bw/day, respectively) as compared to control group (0.75 %). In control recovery group 4.05 % sperms were morphologically abnormal and in high dose recovery group 4.95 %. In addition, historical control data on sperm morphology in two-generation studies on Wistar rats indicate that in F0 generation 5.3 % of sperm heads were abnormal in average. Significant increase of abnormal sperm cells in this case could be due to low percentage of abnormal sperms in control group and is probably not treatment related.

In males, a statistically significant increase in the absolute weight of spleen was observed in low (36 %) and high dose group (53.20 %) as compared to control group. In low dose group and control recovery group absolute spleen weight was comparable (136 % and 132 %, respectively). In high dose group and in high dose recovery group absolute spleen weight was similarly increased compared to control (153 %) what indicates that spleen could be affected by MIT. Also relative spleen weight was increased in high dose group males. Histopathological examination of spleen in high dose group did not reveal any lesions of histopathological significance.

Smear examination of bone marrow revealed hypocellularity, hypercellularity, lymphoid hyperplasia and eosinophilic hyerplasia in both sexes. The effects observed were not considered to be treatment-related.

From this study a NO(A)EL 30.1 mg MIT/kg bw/day was derived (historical control data), LOAEL not being determined (Thor GmbH).

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Toxicity after repeated oral exposure to MIT was tested in rats and dogs.

In 28-day oral toxicity study in rats deaths occured, lethargy, reduction in the weekly body weight gain and feed consumption during the experiment was observed. LOAEL 71.2 mg MIT/kg bw/day was determined based on lethargy and deaths observed at this dose. In this study NOAEL 28.6 mg/kg bw/day was determined.

A subchronic oral toxicity study (drinking water) was conducted in rats. Effects on body weight, food and water consumption were noted at 66 and 94 mg a.i./kg bw/day in males and females, respectively (1000 ppm). **There was no evidence of systemic toxicity or gross and microscopic pathology** at doses up to and including 19-25 mg/kg bw/day (250 ppm), so NOAEL 19-25 mg/kg bw/day was derived in this study.

In a second repeated oral toxicity study in rats NOAEL 30.09 mg/kg bw/day was determined. No adverse effects were observed in this study.

Repeated oral toxicity was assessed in dogs. In both sexes **decreased body weight and food consumption were observed** at 40.6 to 40.9 mg/kg/day (1500 ppm). NOAEL 9.9 mg/kg bw/day was set in males and 11.1 mg/kg/day in females.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

No data available.

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Not relevant for MIT.

4.8.2 Comparison with CLP classification criteria of repeated dose toxicity findings relevant for classification as STOT RE

Not relevant for MIT.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Data are available with MIT by oral and dermal routes and with MIT/CMIT by inhalation. The result indicates that after repeated inhalation exposure to MIT the critical effect will probably be observed at the site of contact. In addition, the effects reported do not appear severe enough to warrant classification. Some effects were considered equivocal and within historical control data. In conclusion, no classification for Specific Target Organ Toxicity (STOT) after repeated exposure (STOT RE) is required.

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

Summary of the Dossier Submitter's proposal

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

In a 28-day oral toxicity study in rats (gavage) deaths occurred and lethargy, reduction in the weekly body weight gain and feed consumption during the experiment was observed. However, gross and histopathological findings observed were not considered treatment related and were recorded either both in control and treatment groups at comparable levels or only in a few animals without any consistent pattern and were in conformity with historical control data. The DS concluded the above effects were spontaneous/incidental findings.

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

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

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

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

Comments received during public consultation

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

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

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

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

Assessment and comparison with the classification criteria

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

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

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

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

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

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

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

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

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

4.9 Germ cell mutagenicity (Mutagenicity)

MIT has been tested for *in vitro* gene mutations in bacteria and mammalian cells and chromosomal aberrations in mammalian cells. *In vivo*, micronucleus assay was performed in mice. Additionally genotoxicity was tested in *in vivo/in vitro* unscheduled DNA synthesis in rat hepatocytes (Tables 14a and 14b).

Table 14a: Summary table of relevant in vitro mutagenicity studies

Test system/ Method Guideline	Organism/ strain(s)	Tested material/ Concentrations tested	Result		Remark	Reference
			+ S9	- S9		
Ames test, OECD 471	*Salmonella typhimurium*, TA 1535, TA	Kordek 573T, 97.5 % active	Not muta-	Not muta-	Toxicity was observed in the definitive assay in all strains at	Sames, J.L. and Streelman

	1537, TA 98, TA 100, TA 102	substance, 5 to 1000 µg/plate	genic	genic	1000 µg/plate with metabolic activation and in strains TA98, TA100 and TA1535 at 500 µg/plate without metabolic activation. In the confirmatory assay toxicity was observed in TA100 at 600 µg/plate with metabolic activation.	D.R. (1999) (A6.6.1/01, Rohm and Haas)
Ames test, OECD 471	*Salmonella typhimurium*, TA 1535, TA 1537, TA 98, TA 100.	Acticide SR 3267 (49 % MIT in water); 8-648 µg Acticide SR3267/plate equal to 3.9 to 317.5 µg MIT/plate	Not muta-genic	Not muta-genic	Toxicity was observed at 648 µg Acticide SR 3267 /plate in direct plate incorporation assay and 72 µg Acticide SR 3267 /plate in pre-incubation method, both without S9-mix.	A 6.6-01 (Thor GmbH)
Gene mutation study (HGPRT) in CHO cells, OECD 476	Chinese Hamster Ovary cells	Kordek 573T, 97.5 % active substance, definitive assay: 0.5, 1.0, 5.0, 10, 15 and 25 µg/ml; confirmatory assay: 5.0, 10, 15, 25 and 40 µg/ml.	Not muta-genic	Not muta-genic	The concentration of 40 µg/ml without S9 activation could not be cloned due to toxicity.	A6.6.3/01 (Rohm and Haas)
Gene mutation study (HPRT) in mammalian cells, OECD 476	Chinese Hamster Ovary cells	Acticide SR 3267 (49 % MIT in water). First test: 0.25-4.0 µg/ml with and without metabolic activation. Second test: 1.0 to 5.0 µg/ml with/ without S9	Not muta-genic	Not muta-genic	No increase in mutant frequencies was observed in the presence or absence of metabolic activation.	A 6.6.3-01 (Thor GmbH)
Cytogenetic study in CHO cells, OECD 473	Chinese Hamster Ovary cells	Kordek 573F, (purity 97.5 % a.s.)Initial test: 0.0785 to 40.0 µg/ml with/ without S9. Confirmatory test: 0.157 to 20.0 µg/ml without S9 and 1.25 to 20.0 µg/ml	Not muta-genic	Not muta-genic	High level of cytotoxicity (decrease of mitotic index or decreased cell count) at highest dose tested *in vitro*. Increase in number of chromosomal aberrations is considered a false negative result, observed at doses that induced cytotoxicity.	A6.6.2/01 (Rohm and Haas)

		with S9.				
Chromosomal aberration study in human lymphocyte cultures, OECD 473	Human lymphocyte culture	Acticide M50, 49.5 % active substance, 2.5, 5 and 10 µg/ml Acticide 50M /plate; equal to 1.3, 2.5 and 5 µg/ml.	Not muta-genic	Not muta-genic	MIT did not induce chromosomal aberrations and mitotic index in the absence or presence of S9 mix (5 and 15 %).	A 6.6.2-01 (Thor GmbH)

Table 14b: Summary table of relevant in vivo mutagenicity studies

Type of test Method/ Guideline	**Species Strain Sex no/group**	**Frequency of application**	**Sampling times**	**Dose levels**	**Results**	**Remarks**	**Reference**
Micronuc-leus in bone marrow erythrocytes, OECD 474 (purity 97.5 % a.s.)	Mouse CD-1, 5/sex/group (7/sex/group in high dose)	single oral dose (gavage)	24 and 48 hr after treatment	0, 10, 50, and 100 mg/kg bw	Not mutagenic	At the highest dose two females showed clinical signs and were found dead 24 hours after exposure.	A6.6.4/01 (Rohm and Haas)
Micronuc-leus in bone marrow erythrocytes, OECD 474 (purity 49.8 % a.s.)	Mouse CRL:NMRI BR, 5/sex/group	single oral dose (gavage)	24 and 48 hr after treatment	0, 100, 150, and 200 mg Acticide SR 3267/kg bw, corresponding to 0, 49.8, 74.4 and 99.6 mg MIT/kg bw	Not mutagenic	Formation of micronuclei was not induced after exposure to MIT.	A 6.6.4-01 (Thor GmbH)
Unscheduled DNA Synthesis, OECD 486 (purity 51.1 % a.s. in water)	Rat, Crl:CD® (SD)IGS, males and females for range-finding assay, males for definitive assay¸4 males/dose; 6 males for high dose	single oral dose (gavage)	2-4 hr and 14-16 hr after treatment	0, 103, 206, or 308 mg a.i./kg	Not mutagenic	Salivation and hypoactivity were observed in treated rats	A6.6.4/02 (Rohm and Haas)

Unscheduled DNA Synthesis, OECD 486 *(CIT/MIT (3:1): 13.9 % w/w, aqueous solution* CIT, 5-chloro-2-methyl-4-isothiazolin-3-one, CAS 26172-55-4: 10.2 % and MIT, 2-methyl-4-isothiazolin-3-one; CAS 2682-20-4: 4.0 %)	Rat, Wistar, males, 6 males/ dose	single oral dose (gavage) of 14 % CMIT/MIT mixture (3:1)	2-4 hr and 12-14 hr after treatment	0, 19 and 60 mg Acticide 14/kg bw, corresponding to 2.64 and 8.34 mg CMIT/MIT (3:1)/kg bw.	Not mutagenic	No increase in unscheduled DNA synthesis was observed.	A 6.6.5-01 (Thor GmbH)

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Genotoxic potential of MIT and MIT technical was evaluated in two Ames tests, gene mutation study (HGPRT) in CHO cells and in chromosomal aberrations study in CHO cells.

A key study on mutagenicity in bacteria was conducetd on *Salmonella typhimurium* strains TA98, TA100, TA1535; TA1537 and TA102 with and without metabolic activation system. At the highest concentrations used cytotoxicity was observed in bacteria exposed to MIT in the presence or absence of S9. MIT did not increase the number of revertant colonies under conditions of this test. Another Ames test was performed with MIT at lower concentrations (0.0001 to 100 µg/plate) and its negative outcome supports the result of the first Ames test. However, concentrations is this test were low and therefore the study is a supportive non-key study (Rohm and Haas).

Ames test was used to test Acticide SR 3267 (49 % MIT) in bacterial systems. Salmonella typhimurium strains TA 98, TA 1537, TA100 and TA 1535 were used in the assay. S. typhimurium stran TA102 or Ecsherichia coli WP2 were not used in the test as it should have been according to the OECD Guideline 471. Bacteria were treated with 8, 24, 72, 216 and 648 µg Acticide SR 3267/plate, corresponding to 3.9, 11.8, 35.3, 105.8 and 317.5 µg MIT/plate, in direct plate incorporation assay and pre-incubation method (1 hour preincubation of bacteria, S9 mix and tested substance). In both experiments number of revertants was slightly increased in TA 1535 in the absence of metabolic activation, however this result was not considered to be positive. Growth inhibition effects of the test substance to the test bacteria in pre-incubation method were visible in the decreasing revertant numbers at concentrations of 216 µg Acticide SR 3267/plate (105.84 µg

MIT/plate) and more, and in the impaired background growth at concentrations > 72 µg/plate (35.28 µg MIT/plate) always without S9-mix for metabolic activation. In direct plate incorporation method growth inhibition effects of the test substance to the test bacteria were visible in the decreasing revertant numbers and in the impaired background growth at concentrations of 648 µg Acticide SR 3267/plate (317.5 µg MIT/plate) always without S9-mix for metabolic activation. Under the test conditions MIT is not mutagenic in *Salmonella typhimurium* strains TA98, TA 100, TA 1535 and TA 1537 with and without metabolic activation (Thor GmbH).

MIT was negative in *in vitro* gene mutation study (HGPRT) in Chinese hamster ovary cells with and without metabolic activation. Concentrations were tested up to the cytotoxic level (Rohm and Haas).

Frequency of HGPRT mutants was evaluated in CHO cells after treatment with 0.25, 0.5, 1, 2 and 4 µg Acticide SR 3267/ml in the first test (corresponding to 0.124, 0.249, 0.498, 0.996 and 1.992 µg MIT/ml) and 1, 2, 4 and 5 µg Acticide SR 3267/ml (corresponding to 0.498, 0.996, 1.992 and 2.49 µg MIT/ml) in the second test, both tests were preformed in the presence and absence of S9 mix. Cytotoxicity was observed at 5.00 µg Acticide SR 3267/ml in Test 2, so cells treated with this concentration were not suitable for examination. Mutant frequencies were increased, but not significantly, in both tests in the presence of S9 mix and in the absence of S9 mix in the first test. Under the test conditions MIT does not induce gene mutations in the cultured human lymphocytes in the presence or absence of metabolic activation system (Thor GmbH).

MIT was also tested in Chinese hamster ovary cells for induction of chromosomal aberrations. The increase in the number of chromosomal aberrations was observed at concentrations of MIT that exerted cytotoxic activity what was indicated by reduction of mitototic index or cell count (27-56 %). The result is considered to be false positive what is supported with the scientific article by Hilliard et al. (1998), that was submitted as a non-key study (Doc IIA, A6.6.2/02). Results of that study demonstrate that numerous compounds induce chromosomal aberrations by secondary mechamisms of cytotoxicity as measured by reduction of cell count or mitotic index (Rohm and Haas).

Acticide M 50 (2.5, 5 and 10 µg/ml, corresponding to 1.3, 2.5 and 5 µg MIT/ml) was tested in *in vitro* mammalian chromosomal aberration test in human lymphocyte culture. Cytotoxicity data of preliminary study showed that 10 µg/ml could be the highest dose used in the main test. Lymphocytes were exposed to MIT for 3 hours with/without S9 (5 %), 30 hours with and without S9 (5 %) and 3 hours with/without S9 (15 %). Acticide M 50 did not induce chromosomal aberrations in short and longer term exposure period. The negative result with metabolic activation system (5% v/v S9 mix) was confirmed by increasing the concentration of S9 to 15 % v/v S9 mix in phase III. The results of the positive controls (cyclophosphamide and mitomycin C) showed an increase in frequency of aberrant cells and demonstrated the sensitivity of the test system (Thor GmbH).

4.9.1.2 *In vivo* data

MIT was tested *in vivo* in CD-1 mouse for the induction of micronuclei formation. At the highest dose tested (100 mg/kg bw) two females were ataxic, passive and exhibited laboured breathing approximately 24 hours after dosing. These animals were found dead

24 hours after dosing. MIT did not the increase the frequency of micronucleated polychromatic erythrocytes in bone marrow of CD-1 mice. Results of tissue distribution study of ^{14}C-RH-573 in the CD-1 mice (Doc IIA, A6.2/03) show that MIT reached the bone marrow tissue and that the highest concentration of MIT was detected 24 hours after exposure (Rohm and Haas).

Incidence of micronuclei was evaluated in bone marrow of CRL:NMRI BR mice after a signle oral gavage dose of 100, 150 and 200 mg Acticide SR 3267/kg bw (corresponding to 49.8, 74.4 and 99.6 mg MIT/kg bw). No clinical signs and no deaths were reported in this study. Slight decrease in a PCE/NCE ratio was noted in high dose females (both time points) and in high dose males (at 24 hours). The positive control induced a significant reduction in the PCE/NCE ratio. In toxicokinetics/metabolism study (A 6.2-01) MIT was determined in bones, therefore exposure of the bone marrow is expected. MIT did not increase the incidence of micronucleated PCE at 24 and 48 hours after single gavage application in mice (Thor GmbH).

Additionally MIT was also negative in the *in vivo/in vitro* unscheduled DNA synthesis in primary rat hepatocytes (Rohm and Haas).

In order to assess the potential of MIT to induce unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure Wistar male rats were exposed to ACTICIDE 14, that contains 14 % of mixture CMIT/MIT (3:1). Rats were administered 19 and 60 mg Acticide 14/kg bw, corresponding to 2.64 and 8.34 mg CMIT/MIT (3:1)/kg bw. No clinical signs and no increase in unscheduled DNA synthesis were observed in exposed animals. Animals were exposed to combination of CMIT/MIT (rats were exposed to 0.67 and 2.64 mg MIT/kg bw). This study is used as a supportive study (Thor GmbH).

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

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

MIT was not genotoxic under tested conditions.

4.9.5 Comparison with CLP classification criteria

Not relevant for MIT.

4.9.6 Conclusions on classification and labelling

MIT is not genotoxic. No classifation is required.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

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

4.10 Carcinogenicity

No data available

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral and dermal

No data available.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

Not relevant for MIT.

4.10.5 Comparison with CLP classification criteria

Not relevant for MIT.

4.10.6 Conclusions on classification and labelling

No classifation is required.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No data were available.

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

4.11 Toxicity for reproduction

The reproductive toxicity of MIT has been investigated in rat and rabbit oral (gavage) developmental studies and in a rat oral (drinking water) multigeneration reproduction study.

Table 15a: Summary table of relevant reproductive toxicity studies

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses	Critical effect	NO(A)EL Parental (mg/kg bw/d)		NO(A)EL F1 (mg/kg bw/d)		NO(A)EL F2 (mg/kg bw/d)		Reference
						m	f	m	f	m	f	
Oral (dietary) Purity: 96-98 %	3-gen, OECD 416	Rat/Crl:CD (SD)IGS BR, M+F, 30/sex/ group	70 days prior to pairing, through mating, gestation, lactation of 2 litters	0, 50, 200, 1000 ppm	See text below	15-19 (200 ppm)	22-26 (200 ppm)	15-19 (200 ppm)	22-26 (200 ppm)	15-19 (200 ppm)	22-26 (200 ppm)	A6.8.2/01 (Rohm and Haas) A6.8.2 (Thor GmbH)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

A rat oral (drinking water) three-generation reproduction study was used to evaluate the effects of MIT on fertility. F_0 males and females received MIT from 70 days prior to pairing, then throughout mating, gestation and lactation of two litters (F_1 and F_2). Animals were dosed with 0 ppm, 50 ppm (4-7 mg/kg bw M, 6-13 mg/kg bw F), 200 ppm (15-19 mg/kg bw M, 22-26 mg/kg bw F) and 1000 ppm MIT (69-86 mg/kg bw M, 93-115 mg/kg bw F). This treatment schedule was repeated on two subsequent generations. The parameters monitored in parents were: clinical signs, body weight, food and water consumption, oestrus cycle, testes weight; and in pups: number and sex, stillbirths/livebirths, presence of gross abnormalities, weight gain, physical or behavioural abnormalities. Histopathology and organ weight investigations were also conducted.

There were no treatment-related mortalities, clinical signs of toxicity, or macroscopic abnormalities. Reproductive performance, parturition and spermatogenic endpoints were unaffected by the test article. Body weight gain, food consumption and water consumption of generation F0 and F1 are presented in the Table 15b.Water consumption was decreased in males at all dose levels. Reduction in water consumption was also observed in females of F0 and F1 generation during gestation and lactation in 200 and 1000 ppm groups. These finding is most likely due to adverse taste or smell of the teste substance. In 1000 ppm group decreased body weight and food consumption were observed and were probably associated with decreased water consumption. No treatment-related systemic or neurological effects were seen in the daily clinical observations or in weekly detailed physical examinations in F_0 and F_1 parental animals at any dose. No test-related macroscopic or microscopic changes neither effects on mean organ weights of F_0 or F_1 were observed at any dose.

Table 15b: Body weight gain, food consumption and water consumption of generation F0 and F1

Parameter	Generation	Control	50 ppm	200 ppm	1000 ppm

			M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body weight gain W 0-18 (males),W 0-10 (females)	% of control	F0	--	--	100	94	98	94	86*	80*
Body weight gain GD 0-20	% of control	F0	--	--	--	102	--	98	--	86*
Body weight gain LD 0-20	% of control	F0	--	--	--	107	--	85	--	137*
Food consumption W 0-18 (males),W0-10 (females)	% of control	F0	--	--	100	90	99	97	94*	93
Food consumption GD 0-21	% of control	F0	--	--	--	102	--	96	--	92*
Food consumption LD 0-21	% of control	F0	--	--	--	98	--	95	--	89*
Water consumption W 0-18 (males),W0-12 (females)	% of control	F0	--	--	91	95	81*	84*	68*	68*
Water consumption GD 0-21	% of control	F0	--	--	--	98	--	80*	--	59*
Water consumption LD 0-21	% of control	F0	--	--	--	99	--	89*	--	75*
Body weight gain W 18-36 (males), W 18-28 (females)	% of control	F1	--	--	101	106	95	101	88*	99
Body weight gain GD 0-20	% of control	F1	--	--	--	105	--	97	--	86*
Body weight gain LD 0-20	% of control	F1	--	--	--	107	--	85	--	137*
Food consumption W 0-18 (males),W0-12	% of control	F1	--	--	99	100	97	103	88*	91*

Parameter		**Generation**	**Control**		**50 ppm**		**200 ppm**		**1000 ppm**	
			M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
(females)										
Food consumption GD 0-21	% of control	F1	--	--	--	100	--	100	--	91*
Food consumption LD 0-21	% of control	F1	--	--	--	98	--	98	--	88*
Water consumption W 0-18 (males),W0-12 (females)	% of control	F1	--	--	100	96	91*	89*	69*	68*
Water consumption GD 0-21	% of control	F1	--	--	--	95	--	81*	--	59*
Water consumption LD 0-21	% of control	F1	--	--	--	95	--	91*	--	73*

*-Significantly different from the control group at 0.05 using Dunnett's test

Decreased water consumption was noted in F_1 males and females at the 200 and 1000 ppm dose levels during the pre-breeding period. Water consumption was decreased at all dose levels for the F_1 generation during the week following weaning (post-natal days 21-28) when the animals were housed by litter. Decreased water consumption was noted in F_2 females at the 200 and 1000 ppm dose levels during the pre-breeding period. Decreased water consumption was not indicative of systemic toxicity, but most likely due to aversion to the taste and/or smell of the test article, an irritant. Decreased body weight of F1 and F2 pups was observed on PND 7, 14 and 21 (Table 15c). No treatment-related systemic or neurological effects were seen in the daily clinical observations or in detailed physical examinations (PND 1, 4, 7, 14 1and 21) in F_1 and F_2 pups at any dose. In the 1000 ppm group of P_1 pups delay in the mean day of acquisition of balanopreputial separation and of vaginal patency was observed. The mean day of acquisition of balanoperputial separation and vaginal patency were within the historical data range. However, these effects were not a direct result of test article but related to a decrease in mean body weights of pups at day of acquisition. Anogenital distances for the F_2 pups were unaffected by treatment with the test article.

Table 15c: Mean body weight of F 1 and F2 pups

Mean body weigh compared to the control (%)								
F1	Males				Females			
	0 ppm	50 ppm	200 ppm	1000 ppm	0 ppm	50 ppm	200 ppm	1000 ppm

PND 1	100	96	99	94*	100	98	100	94
PND 7	100	95	99	90*	100	99	99	91*
PND 14	100	95	99	88*	100	99	99	87*
PND 21	100	95*	96	84*	100	97	96	84*
F2	Males				Females			
PND 1	100	96	96	100	100	96	97	97
PND 7	100	97	99	99	100	96	98	97
PND 14	100	99	100	93*	100	99	99	92*
PND 21	100	98	99	87*	100	97	100	86*

*-Significantly different from the control group at 0.05 using Dunnett's test

No microscopic changes were observed in the brains of pups of either the F_1 or F_2 generation exposed to 1000 ppm MIT *in utero*, through nursing, during lactation or in the drinking water following weaning.

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 15d: Summary table of relevant development toxicity studiy

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses	Critical effects dams / fetuses	NOAEL maternal toxicity	NOAEL Teratogenicity Embryotoxicity	Reference
Oral (gavage)	Develop-mental toxicity OECD 414 GLP	Rat/Crl:CD (SD)IGS BR, F, 25/group	GD 6-19	0, 5, 20 and 60/40 mg/kg bw/day Purity: 96-98 % Kordek 573 F (51.4 % MIT in water)	Maternal toxicity: at 60/40 mg/kg bw/day 3 animals were found dead, 2 were euthanized. Clinical findings in survivors: rales, gasping and labored respiration. At necropsy red areas in the glandular portion of the stomach and lung findings were observed. ↓ mean body weight gain (gestation days 6-9) (17.5 %), ↓ mean net body weight gain (28 %) and ↓ food consumption (gestation days 6-9) (16.7 %) were noted. No maternal effects at 5 or 20 mg/kg bw/day. Foetal toxicity: no effects on intrauterine growth and survival, number of fetuses/litter, number of resorptions, fetal body weight or sex ratio. There were no treatment- related external, soft-tissue, head or skeletal malformations, variations, or developmental retardations observed at any dose level.	20 mg/kg bw/day	40 mg/kg bw/day	A6.8.1a/01 (Rohm and Haas)
Oral (gavage) (purity 49,8% a.s.)	Develop-mental toxicity OECD 414 GLP	Rat/Crl (WI)BR, F, 25/group	GD 6-15	0, 67, 100 and 150 mg Acticide SR 3267/kg bw/day, corresponding to 0, 33.4, 50 and 75 mg MIT/kg bw/day	Maternal toxicity: at 50 and 75 mg/kg bw/day ↓ mean body weight gain (16 and 30 %, respectively) and ↓ food consumption were noted during treatment. Foetal toxicity: At 75 mg MIT/kg bw/day: ↑ incidence of dilated cerebral ventricles (12.3 % fetuses,14/22 litters), ↑ unossified metatarsals (78 % fetuses, 21/22 litters). At 50 and 75 mg/kg bw/day: ↑ (72 % foetuses, 21/22 litters, at 75 mg/kg bw and 78 % foetuses, 21/21 litters, at mg/kg bw) unossified cervical vertebral bodies. No effects on intrauterine growth and survival, number of fetuses/litter, number of resorptions, fetal body weight or sex ratio. At 33.4 mg/kg bw/day: no effect	33.4 mg/kg bw/day	33.4 mg/kg bw/day	A6.8.1-02 (Thor GmbH)
Oral (gavage)	Develop-mental toxicity OECD 414 GLP	Rabbit/New Zealand White, F, 25/group	GD 6-28	0, 3, 10 and 30 mg/kg bw/d Purity: 96-98 % Kordek 573 F (51.4 % MIT in water)	Maternal toxicity: at 30 mg/kg bw/day ↓ defecation (4/25), dark red areas in the stomach (6/25), mean body weight loss during days 6-9, ↓ food consumption on days 6-9, 9-12 and 12-21. No maternal effects were observed at 3 or 10 mg/kg bw/day. Foetal toxicity: no effects at any dose level on intrauterine growth and survival, number of fetuses/litter, number of resorptions, fetal body weight or sex ratio. There were no treatment- related external, soft-tissue, head or skeletal malformations, variations, or developmental retardations observed at any dose level.	10 mg/kg bw/day	30 mg/kg bw/day	A6.8.1b/01 (Rohm and Haas) A6.8.1-01 (Thor GmbH)

In the rat teratogenicity study, females received MIT from days 6 to 19 of gestation. On days 6-9 majority of animals received 60 mg/kg bw/day. This dose exceeded maximum tolerated dose and therefore the dose was lowered to 40 mg/kg bw/day. Three animals in the 40/60 mg/kg bw/day dose group were found dead, 2 were euthanized in the moribund state. Clinical signs of the females either found dead or killed in extremis included: rocking, lurching or

swaying while ambulating, hypoactivity, rales, gasping, labored respiration, decreased defecation, red material around the nose, mouth and/or eyes. In survivors rales, gasping and labored respiration were observed. Red areas in the glandular portion of the stomach and lung findings (dark red discoloration of the lungs, dark red areas in the lungs and/or lungs not fully collapsed) were detected at necropsy. Reductions in body weight gain and food consumption were reported in this group. No test article-related effects on mean body weight, body weight gain, gravid uterine weight, food consumption and internal findings at necropsy were noted in the 5 and 20 mg a.i./kg/day groups.

MIT did not affect the number of corpora lutea or implantations, the number of resorptions, foetal body weight or sex ratio. No treatment-related external, visceral or skeletal malformations or variations were observed in the foetuses. A NOAEL for maternal toxicity of 20 mg/kg bw/day was determined, based on animal deaths, clinical signs, reduced body weight gain and necropsy findings at 40/60 mg/kg bw/day. The NOAEL for developmental toxicity was 40 mg/kg bw/day; the highest dose tested (Rohm and Haas).

In the second rat study females were gavaged 0, 67, 100 and 150 mg Acticide SR 3267/kg bw/day, corresponding to 0, 33.4, 49.8 and 75 mg MIT/kg bw/day from days 6 to 15 of gestation. No maternal deaths and clinical signs were observed in control and treated groups. Body weight gain of dams was significantly and dose-dependently reduced in animals treated with 100 and 150 mg Acticide SR 3267/kg bw/day. In these groups food consumption decreased significantly. Body weight gain during the post-treatment period and total body weight gains during the pregnancy were similar in all experimental groups. There was no autopsy finding of reaction to the treatment in any dose groups.

There were no significant differences in the number of the corpora lutea, implantations and viable foetuses among the examined groups and in the embryonic deaths and foetal death either. There were no significant or dose-related increases in pre- and post-implantation loss in any of the treated groups. Mean foetal body weights and placental weights were unaffected by maternal treatment with ACTICIDE SR 3267. Foetal visceral examination revealed significant increase in number of minor anomaly, dilated cerebral ventricles, at 150 mg/kg bw/day dose group. Number of the visceral variations decreased significantly in fetuses of mothers treated with at 150 mg/kg bw/day.

Regarding skeletal anomalies the number of unossified cervical vertebral bodies was significantly increased at the 100 mg/kg (76 % foetuses, 20/21 litters) and 150 mg/kg bw/day dose levels (72 % foetuses, 21/22 litters). Number of unossified metatarsals was significantly higher in the 150 mg/kg dose group (78 % foetuses, 21/22 litters) than the control value. Delay in ossification is probably related to decreased body weight gain of dams. Significant differences were detected between the control group and 67 mg/kg dose group in incidence of the supernumerary rib without biological significance.

In this study LOAEL 50 mg MIT/kg bw/day and NOAEL 33.4 mg/kg bw/day were derived for developmental effects based on increased incidence of unossified cervical vertebral bodies. LOAEL 50 mg MIT/ kg bw/day and NOAEL 33.4 mg MIT/kg bw/day was derived for maternal toxicity based on decreased body weight gain during gestation (Thor GmbH).

In the rabbit teratogenicity study MIT was administered during gestation days 6 to 28, the animals were sacrificed on day 29. One dam in the high dose group (30 mg/kg bw/day) aborted

on day 25 and in the mid-dose group (10 mg/kg bw/day) one dam was found dead on day 19, likely due to the intubation error. At 30 mg/kg bw/day the following treatment-related effects were observed: decreased defecation (4/25, beginning on gestation day 7), dark red areas in the stomach (6/25), mean body weight loss during gestation days 6-9 and reduced mean food consumption during gestation days 6-9, 9-12 and 12-21. In the female that aborted in the high dose group substantial loss in body weight (22 %) and decreased food intake were observed after beginning of the treatment. At necropsy dark red and white areas were observed in the lining of the stomach. Regarding historical data abortions in control populations are not so rare; therefore this single abortion was not assigned to the tested substance.

The numbers of corpora lutea, implantations, pre- and post-implantation losses, number of foetuses per litter and viable foetuses, the mean foetal and placental weights were unaffected by exposure to MIT.

No treatment-related external malformations or developmental variations were noted at any dose level. No evidence of developmental toxicity of MIT was observed at doses up to and including 30 mg a.i./kg/day (highest dose tested). Based on the results of this study, a dose level of 10 mg a.i./kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. A dose level of 30 mg a.i./kg/day was considered to be the NOAEL for developmental toxicity.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

No data avialable

4.11.4 Summary and discussion of reproductive toxicity

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

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

No effects on fertility and sexual function in rats were observed. Reduced body weight gain in parents and offspring, reduced food intake. Based on the results of the relevant reproductive

toxicity studies, the highest dose tested 1000 ppm (69-93 mg/kg/day) was not toxic for reproduction. However, at 1000 ppm the following test substance-related effects were observed:

(1) Decreased mean body weight gains in males and females during the first one-to-five weeks of each generation and during the middle and/or late parts of gestation and lactation; decreased mean body weights beginning at week 2 or 3 and continuing throughout the remainder of the generation (F0) or throughout the generation (F1). (2) Decreased food consumption throughout each respective generation (males); Decreased food consumption throughout the pre-breeding period and during middle-to-late gestation and middle-to-late lactation (F0 females); Decreased food consumption throughout the pre-breeding period and gestation periods and during middle-to-late lactation (F1 females); decreased food efficiency during the first four or five weeks of the study (F0 only). This finding was most likely associated with decreased water consumption. (3) Decreased mean offspring body weights in the latter part of both the F1 pre-weaning period (post-natal days 7-21) and the F2 pre-weaning period (post-natal days 14-21).

Based on these findings 200 ppm (15-22 mg/kg/day for the F_0 pre-mating period and 19-26 mg/kg/day for the F_1 pre-mating period) is considered a NOAEL for parental toxicity and for neonatal toxicity.

4.11.5 Comparison with CLP classification criteria

Not relevant for MIT.

4.11.6 Conclusions on classification and labelling

No classifation is required.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

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

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

No effects on fertility and sexual function in rats were observed in a three-generation reproductive toxicity study in rats. Reduced body weight gain and reduced food intake were observed in parents and their offspring. Based on the results of the relevant reproductive

toxicity studies, the highest dose tested 1000 ppm (69-93 mg/kg/day) was not toxic for reproduction. However, the DS reported the following test substance-related effects at 1000 ppm MIT:
(1) Decreased mean body weight gains in males and females during the first one-to-five weeks of each generation and during the middle and/or late parts of gestation and lactation; decreased mean body weights beginning at week 2 or 3 and continuing throughout the remainder of the generation (F0) or throughout the generation (F1).
(2) Decreased food consumption throughout each respective generation (males); Decreased food consumption throughout the pre-breeding period and during middle-to-late gestation and middle-to-late lactation (F0 females); Decreased food consumption throughout the pre-breeding period and gestation periods and during middle-to-late lactation (F1 females); decreased food efficiency during the first four or five weeks of the study (F0 only). This finding was most likely associated with decreased water consumption.
(3) Decreased mean offspring body weights in the latter part of both the F1 pre-weaning period (post-natal days 7-21) and the F2 pre-weaning period (post-natal days 14-21).

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

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

Comments received during public consultation

One manufacturer agreed that classification of MIT for reproductive toxicity is not required.
One MS commented that changes in sperm parameters were observed in a repeated dose study (reduced sperm motility and sperm heads). Although these changes were within the relevant historical control range, the MS requested that these findings are included in the discussion on reproductive toxicity (fertility).

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

Additional key elements

In response to the request to include the information on sperm parameters from the repeated dose study in the discussion on reproductive toxicity, the DS provided the following update of relevance to Section 4.11.4 of the CLH report:

In a rat 90 day oral study, changes in sperm parameters were observed in males exposed to MIT (Tables 1 and 2). Sperm motility was not affected by MIT treatment, except in the recovery group, where some reduction was observed. This reduction was within historical control data (mean 93.4±2.17, max 96.6, min 86.5). Dose dependent reduction in the number of testicular sperm heads in testes was observed in animals treated with MIT. Although significantly reduced, the values were within the historical control data range (mean 134.18±15.23, max 173.75, min 92.5). In the rat multi-generation study, no effect on sperm count was observed after exposure to higher concentrations of MIT (60/40 mg/kg bw/day).

The reduction in epididymal sperm count is considered not to be biologically relevant taking into account no change in testes weight and no histopathological changes observed, a reduction within the historical data range (mean 1208.34±168.65, max 1592, min 805), absence of this effect in the recovery group and in the reproduction toxicity study, where animals were exposed to higher doses of MIT. Statistically significant increase in per cent of abnormal sperm samples obtained from cauda epididymis in all treated groups (2.15, 2.35 and 2.80% in animals treated with 7.5, 15 and 30 mg/kg bw/day, respectively) was

reported compared to controls (0.67%). In the control recovery group, 4.05% of sperm were morphologically abnormal; in the high dose recovery group the figure was 4.95%. Additionally, historical control data on sperm morphology in two-generation studies on Wistar rats indicate that in the F0 generation 5.3% of sperm heads were abnormal on average. Significant increase of abnormal sperm cells in this case could be due to low percentage of abnormal sperm in the control group and is probably not treatment related.

Historical control data were submitted from the laboratory where the study was performed, for the strain tested in the 90 day study. The data were derived from two-generation studies performed within two years of the respective study. Male rats were involved in the study for 120-135 days.

The table below presents sperm parameters in MIT treated rats (90-day study).

Parameter	**0 mg/kg**		**7.5 mg/kg MIT***		**15 mg/kg MIT***		**30 mg/kg MIT***	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Motility count (%)	93.0	1.59	92.5	1.47	92.6	0.77	92.5	1.21
Testicular sperm head count (millions/g)	130.625	8.990	121.938↓	5.594	114.75↓↓	5.865	106.458↓↓	7.844
Epididymal sperm count (millions/g)	1355.35	90.98	1371.90	87.91	1438.30	172.52	1371.17	148.66
Abnormal sperm (%)	0.67	0.66	2.15↑	1.29	2.35↑↑	1.25	2.80↑↑	1.09

↓ - significantly lower than control ($p \leq 0.05$), ↓↓ - significantly lower than control ($p \leq 0.01$),
↑- significantly higher than control ($p \leq 0.05$), ↑↑ - significantly higher than control ($p \leq 0.01$)
* Animals were dosed with Acticide (50.7% MIT).

The table below presents sperm parameters in 14 days recovery groups of MIT treated rats (90-day study).

Parameter	**0 mg/kg bw/day**		**7.5 mg/kg MIT* RAC Correction**: 30 mg/kg/day**	
	Mean	**SD**	**Mean**	**SD**
Motility count (%)	92,9	0,77	91,3↓	1,68
Testicular sperm head count (millions/g)	120,875	10,401	133,688	26,320
Epididymal sperm count (millions/g)	1345,80	40,72	1335,85	67,87
Abnormal sperm (%)	4,05	1,46	4,95	1,19

* Animals were dosed with Acticide (50.7% MIT).
** The table provided by the DS seems to have had the wrong dose level indicated for the treated animals. The text that accompanied the table described these data as coming from the high dose group, not the lowest.

In response to the request from the second MS to check consistency of the data in the report, the DS explained that the text corresponding to the second teratogenicity study (p.65 last paragraph) should read as follows:

In the second rat study females were gavaged with 0, 33.4, 50 and 75 mg MIT/kg bw/day from days 6 to 15 of gestation. No maternal deaths and clinical signs were observed in control and treated groups. Body weight gain of dams was significantly and dose-dependently reduced in animals treated with 50 and 75 mg MIT/kg bw/day. In these groups, food consumption decreased significantly. Body weight gain during the post-

treatment period and total body weight gains during the pregnancy were similar in all experimental groups. There was no autopsy finding of reaction to the treatment in any dose groups. In addition, according to the DS:

- There were no significant differences in the number of the corpora lutea, implantations and viable foetuses among the examined groups or in the embryonic deaths or foetal deaths. There were no significant or dose-related increases in pre- and post-implantation loss in any of the treated groups. Mean foetal body weights and placental weights were unaffected by maternal treatment with MIT. Foetal visceral examination revealed significant increase in the incidence of a minor anomaly, dilated cerebral ventricles, at 75 mg MIT/kg bw/day dose group.

- The number of the visceral variations decreased significantly in fetuses of mothers treated with 75 mg MIT/kg bw/day. Regarding skeletal anomalies, the number of unossified cervical vertebral bodies was significantly increased at 50 mg/kg (76% foetuses in 21/21 litters) and 75 mg/kg bw/day dose levels (72% foetuses in 21/22 litters). The number of unossified metatarsals was significantly higher in the 75 mg/kg dose group (78% foetuses in 21/22 litters) than in controls.

- Delay in ossification is probably related to decreased body weight gain of dams. In this study, LOAEL 50 mg/kg bw/day and NOAEL 33.4 mg/kg bw/day were derived for developmental effects based on increased incidence of unossified cervical vertebral bodies. LOAEL 50 mg/kg bw/day and NOAEL 33.4 mg/kg bw/day were derived for maternal toxicity based on decreased body weight gain during gestation.

Assessment and comparison with the classification criteria

Sexual function and fertility

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

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

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

However, changes in sperm parameters were observed in males Wister rats in a 90 d oral toxicity study. The rats were administered 0, 7.52, 15 or 30 mg/kg MIT (50.7% MIT in water), by gavage. In the high dose group, sperm motility was reduced. There was also a dose-dependent reduction in the number of testicular sperm heads in treated animals. For both effects, the results were within the historical control data range. There were no histopathological changes, no reduction in epididymal sperm count and no changes in testis weight. In all treatment groups, a statistically significant increase in mean percent of morphologically abnormal sperm cells from the cauda epididymis was observed (0.67 (standard deviation 0.66), 2.2 (SD 1.29), 2.35 (SD 1.25) and 2.80% (SD 1.09) at 0, 7.5,

15 and 30 mg/kg MIT, respectively). The DS did not elaborate on what was meant by abnormal sperm cells (i.e. tails or heads). The DS indicated that 4.05% and 4.95% of sperm cells were morphologically abnormal in the control 28 day recovery and high dose recovery groups, respectively. Given this, and the very flat dose-response observed at 90 days, it therefore seems likely that the observed effect was a consequence of the concurrent control value rather than due to dosing with MIT. Historical control data from 2-generation studies performed in Wister rats by the same laboratory further support this conclusion. The mean value was 5.3%.

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

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

Developmental toxicity

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

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

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

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

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

In the second study, female rats were exposed by gavage to 0, 33.4, 49.8 and 75 mg MIT/kg bw/d on GD 6-15. No maternal deaths or clinical signs were observed. At 49.8 and

75 mg/kg bw/d, there was a significant and dose-dependent reduction in bodyweight gain of dams and a significant decrease in food consumption.

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

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

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

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

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

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

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

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

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

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

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

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No neurotoxicity studies were perfomed with MIT. However, recently and article on the effect of MIT on isolated rat neurons in culture was published. The article reports that MIT was

cytotoxic for isolated neuron cells. Despite defficiences in the study performed and the fact that the *in vitro* study on isolated organ can not be extrapolated to *in vivo* system an extensive explanation of MIT not showing any sings of neurotoxicity is given in Competent Authority Report (A6.9 of CAR). In repeated dose studies, developmental studies and reproductive toxicity study with MIT **no clinical signs and pathological examinations indicated neurotoxic potential of MIT**. Additionally, in 90-day oral toxicity study in rats **no signs of neurotoxicity were observed** in detailed clinical observations and functional observational battery (FOB).

An extensive set of health effect studies have been conducted in various laboratory animal models with several isothiazoline molecules (i.e. biocidal actives), including MIT, and there **is no evidence of *in-vivo* neurotoxicity with any actives within the isothiazolone family.**

4.12.1.2 Immunotoxicity

No data avialble.

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

No data avialble.

4.12.2 Summary and discussion

No immunotoxicity and neurotoxicity studies were perfomed with MIT. But there are some data in litertature on neurotoxicity (see section 4.12.1.1 of this report).

4.12.3 Comparison with CLP classification criteria

Not relevant for MIT.

4.12.4 Conclusions on classification and labelling

No classification required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of methylisothiazolinone (MIT) were assessed in the Competent Authority Report (CAR) regarding the Directive 98/8/EC and shall be included in Union list of active substances approved for use in biocidal products according Regulation (EU) No. 528/2012. The evaluation based on the two dossiers of two different applicants (Rohm and Haas as well as Thor GmbH) and brief overview of the environmental fate properties is given here.

The summaries included in this proposal are copied from the CAR. For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarised in a table. Detailed information is only included for the key study used to derive the classification. References to individual studies are not included. For more details the reader is referred to the CAR.

5.1 Degradation

5.1.1 Stability

Abiotic degradation of MIT in aqueous media occurs at a moderate rate and is significantly slower than aquatic biodegradation.Thus the primary route of dissipation in the environment is biological.

Hydrolytic degradation

Results of aqueous hydrolysis studies with MIT are summarised in Table 16a.

Table 16a: Results of the hydrolysis studies

Guideline/ Test Method	**Temp [°C]**	**pH**	**Initial TS conc. [µg/mL]**	**Reaction Rate Constant k [hr^{-1}]**	**DT_{50} [d]**	**Coefficient of Correlation (r^2)**	**Reference (Doc III-A)**
OECD 111 US EPA 161-1	25	4	13.3	ND (stable)	ND	NA	Marx, M, Castle, S, and Shepler, K. (1992) (A7.1.1.1.1/01, Rohm and Haas)
		7	9.9	ND (stable)	ND	NA	
		9	10.1	ND (stable)	ND	NA	
OECD 111	50	4	7.78	ND (stable)	ND	NA	A7.1.1.1.1-01 (Thor GmbH)
		7	9.09	ND (stable)	ND	NA	
		9	8.90	ND (stable)	ND	NA	
OECD 111	37	1.2	6.9	ND (stable)	ND	NA	Non-key study (Thor GmbH)
EPA 161-1	25	5	1.8	ND (stable)	ND	NA	Non-key study (Thor GmbH)
		7	1.8	ND (stable)	ND	NA	
		9	1.8	ND (stable)	ND	NA	

Rohm and Haas

In the study A7.1.1.1.1/01 no significant hydrolysis of MIT was observed in pH 5, 7, and 9 buffers under dark conditions as the compound was stable for more than 720 hours at 25ºC. The study was perfomed with ^{14}C-MIT with ^{14}C-label in the 4 and 5 positions. Radioassay was performed using a Beckman liquid scintillation counter. The material balance was determined by radioassaying aliquots of the hydrolysis solution at each sampling time. Recovery of ^{14}C-activity from quantitation was 99.3 ± 4.6 %, 100.3 ± 3.6 %, and 99.3 ± 1.5 % for pH 5, 7, and 9, respectively. Temperature of the test system varied more than 2 ºC during the test. Guideline OECD 111 requires that the temperature is kept constant within a range of ± 0.1 ºC. However, the observed temperature variations are not expected to affect the conclusion that MIT is hydrolytically stable in the pH range 5-9.

Thor GmbH

In the study A7.1.1.1.1-01 the test substance MIT was stable in sterile aqueous media at pH 4, pH 7 and pH 9 over a period of 5 days at 50ºC in the dark. Since less than 10% decline in the MIT concentration at any pH was observed after 5 days (10% hydrolysis at 50ºC would correspond to a degradation half-life at 25ºC of > 1 year), the chemical was considered to be hydrolytically stable. Analysis of MIT was carried out by HPLC. LOD and LOQ of MIT in this system were determined to

be 0.023 and 0.034 mg/L, respectively. The HPLC method was validated with regard to peak symmetry, precision (repeatability), peak resolution and linearity. Guideline OECD 111 requires the rate constant for hydrolysis to be determined in duplicate for each temperature and pH level. Performance of the test with a single replicate as was the case in this study results in a less reliable study. However, this deviation is not expected to affect the conclusion that MIT is hydrolytically stable at pH 4, 7 and 9.

Photochemical degradation in water

Aqueous photolysis of MIT is moderately fast. Table 16b summarizes results of aqueous photolysis studies with MIT.

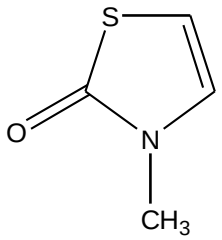
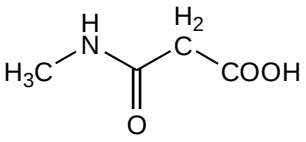
Table 16b: Results of the photolysis studies

Guideline / Test Method	**Initial TS Conc. [µg/mL]**	**Total Recovery of TS (% applied)**	**Photolysis Rate Constant (k^c_p) [day^{-1}]**	**Direct Photolysis Rate Constant (K_{pE})**	**Reaction Quantum Yield (Φ^c_E)**	**Half-Life [d]**	**Reference**
US EPA 161-2	10.8	97.5±5 Light: 96±6.1 Dark: 99±5.3	Light: 0.062 Dark: 0.0016	Not determined since no actinometer study was performed	Not determined since no actinometer study was performed	Light 11.1 Dark 425	Shepler, K.. (1995) (A7.1.1.1.2/01,Rohm and Haas)
US EPA 161-2	2.0	95.8 ± 3.1	Light 0.038 Dark ND	Not determined since no actinometer study was performed	Not determined since no actinometer study was performed	Light 18.2 Dark stable	A7.1.1.1.2-01 (Thor GmbH)
- only metabolite characterisation	17 and 170	94.8-102.7	ND	ND	ND	ND	Non-key study (Thor GmbH)
- only metabolite characterisation	200	94.8-101.0	ND	ND	ND	ND	Non-key study (Thor GmbH)

Rohm and Haas

Metabolism in the photolysis study involved ring cleavage. In addition to CO_2 (2.7 %), several degradates have been formed, with two of them in a quantity above 10 %. The first major degradate increased to a maximum of 38 % at the end of the study. This degradate was characterized as mixture of N-methyl malonamic acid, N-methyl acetamide and N-methyl oxamic acid with N-methyl malonamic acid as primary component. The other major degradate reached a maximum of nearly 4 0 % at the end of the study. This degradate was identified by mass and NMR spectroscopy as 3-methyl-4-thiazolin-3-one.

Figure 5.1.1c Proposed structure metabolites from photolysis study > 10 %

	
3-methyl-4-thiazolin-3-one 40 %	N-methyl malonamic acid 39 %

Thor GmbH

The levels of radioactivity detected as degradates in the aqueous buffer solution and as volatile degradates increased parallel to the decrease of MIT levels. Three major degradates detected were designated as Unknown 8, Unknown 10 and Unknown 4. The Unknown 8 reached 27 % of the TAR after 25.3 days. The investigation of Unknown 8 by mass spectrometry indicated that it is a rearrangement product of MIT. Unknown 10 and Unknown 4 similarly increased through the study, and reached 16 and 11% of the TAR at 25.3 days, respectively.

For the purpose of identification of photodegradation products, an aqueous photodegradation study of ^{14}[C]-MIT was conducted under buffered (pH 7) and artificial sunlight conditions at 25 °C for one day, for the identification of degradation products of MIT. Results demonstrated that photolysis of MIT occurred and that degradation products were present in irradiated samples, with a similar pattern observed in the study by Purser (1998). However, no information with respect the structure of MIT photolytic degradation products could be obtained using ^{1}H and ^{13}C-NMR and LC-MS/MS. The formation of polar MIT degradates was confirmed by HPLC-UV. They could not be determined by LC-MS/MS due to their assumed low masses. The ^{1}H and ^{13}C-NMR analysis could not provide further information about the less polar degradate of MIT, which was found to be instable during the analytical procedure.

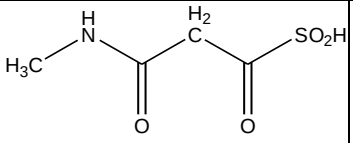
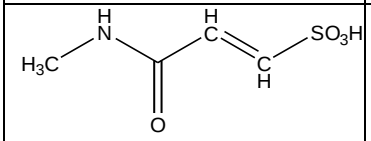
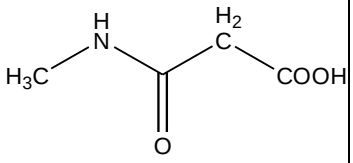
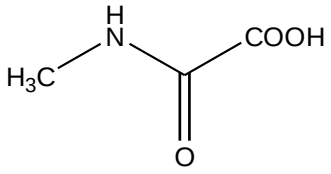
In a next study, the elucidation of MIT photodegradation products was attempted. Radiation simulated the solar spectrum at noon at 40-50° N latitude during 7 days (24-hour day). The photolytic degradation half-life for MIT was determined to be 0.4 days. One major transient degradation product was detected (DegM1) and was found to reach 43.9% of the TAR after one day. In the HPLC analysis, its elution occurred after the parent substance, indicating that this degradation product is less polar than MIT. It was also photolytically unstable (DT_{50} 4 days). It is thought that the first photolytic degradation step is a transformation from MIT to DegM1, which degrades further to other degradation products. Three less stable degradation products were detected at amounts smaller than 10% of the TAR: DegM2, DegM3 and DegM4. In view of their very short retention times, it can be assumed that these degradation products are very polar. From dark control experiments, it can be concluded that MIT and all its degradation products are stable under dark conditions. The structure of DegM1 was not identified. Accurate mass measurements demonstrated that it is an isomer of MIT. DegM1 was the main degradate of MIT detected in this study. LC-MS peaks did not match with the reference compounds included in this study, including N-methyl malonamic acid.

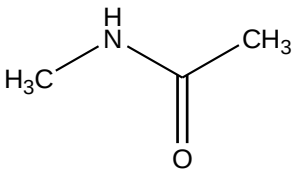
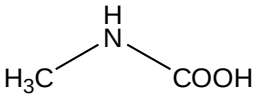
Air phototransformation

Rohm and Haas

The phototransformation rate constants and half-lives were calculated using structure activity relationship (SAR) methods. The rate constant, k, was calculated from the OH- and NO_3 radical reaction processes and the resulting rate constant used to calculate the half-life.
The calculated half-lives for both OH and NO_3 radical reactions for MIT were 16.6 and 29.9 hours, respectively. The calculation for reaction with OH-radicals is in accordance with Equation 28 in the TGD (2003). For the observed metabolites and degradation products of MIT the half-lifes range from 25.2 to 31.8 hours (see Table 17c). MIT and its photodegradation products are rapidly degraded in air during the daylight. Due to the physical-chemical properties of MIT the concentration of MIT in the troposphere is expected to be low. As a result of the short half-life and the low potential for partitioning into the troposphere, it is unlikely that MIT (or its photodegradation products) will be significantly transported through the troposphere or significantly influence global warming.

Table 16c Results from air phototransformation calculations

Guideline	**Method**	**Compound**	**Half-Life [h]**	**Reference**
Technical Guidance Document, Chapter 3, Section 7.3.1	Calculation	MIT	16.6	**Guo,I (2003)** **(A7.3.1/01, Rohm and Haas)**
		 2-(methylcarbamoyl)-1-oxoethane sulfinic acid	25.2	
		 2-(methylcarbamoyl)-ethene sulfonic acid	27.1	
		 N-methyl malonamic acid	31.6	
		 N-methyl oxamic acid	31.7	

		 N-methyl acetamide	31.6	
		 N-methyl carbamate	31.8	

Thor GmbH

The rate constant for phototransformation of MIT in air was estimated using the AOPWIN QSAR software (reference A.7.3.1-01). A tropospheric half-life of 0.6 days (14.3 hours) was calculated for reaction of OH-radicals with MIT, assuming 24 hours of sunlight, 25 °C, and an OH-radical concentration of $5 \cdot 10^5$ cm^{-3}. The OH-radical concentration of $5 \cdot 10^5$ cm^{-3} used for the calculation of the half-life presented above is the default value given in the TGD. The half life contained in the report as calculated is based on an OH-radical concentration of 1.5 10^6 molecules·cm^{-3} and resulted in a half-life of 4.78 hours. The reaction with ozone was estimated to be slow as compared to the reaction with OH-radicals, and therefore was not considered in the calculation of the overall half-life. MIT reaching the air is rapidly degraded. However, MIT is generally not expected to volatilise or partition to air to any relevant extent.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available.

5.1.2.2 Screening tests

Results of ready biodegradation tests with MIT are summarised in Table 17.

Table 17: Results from the ready biodegradation tests with MIT

Guideline / Test method	**Test type**	**Test para-meter**	**Inoculum**			**MIT conc. [mg/l]**	**Degradation**		**Reference**
			Type	**Conc.**	**Adap-tation**		**Incubation period**	**Degree [%]**	
OECD 301B Modified Sturm test	Ready biodegra dability	CO_2	WWTP activated sludge	30 mg d.w./l washed sludge	no	0.01	28 days	54.1	Bashir, M. (1998) (A7.1.1.2.1/01 ,Rohm and Haas)
						0.03	28 days	55.8	
						0.1	28 days	47.6	
OECD 301D Closed Bottle Test	Ready biodegra dability	O_2	WWTP activated sludge	10^4-10^6 CFU/mL	no	10	28 days	0	A7.1.1.2.1-01 (Thor GmbH)
OECD 301A	Ready	DOC	WWTP	10^7-10^8	no	10	36 days	17	Non-key

DOC Die	biodegra		activated	CFU/mL		20	36 days	12	study

Rohm and Haas

In this study ready biodegradation was tested at three concentrations. MIT rapidly biodegrades up to 48-56 %, but based on current guidelines, it cannot be classified as readily biodegradable since it does not biodegrade to 60 % and does not satisfy the 10-day window requirement. Because of the toxicity of MIT, lower concentrations that recommended by the OECD guideline have been used in this biodegradability test.

Thor GmbH

In view of the outcomes of the tests, MIT is found to be not readily biodegradable. No biological degradation of MIT was observed within 28 days in activated sewage sludge from a municipal sewage treatment plant. No explanation was given for the high oxygen demand in the inoculum control. The high depletion of oxygen in the control (3.5 mg/L instead of less than 1.5 mg/L) should have been justified, The initial oxygen content appears also higher than the recommended value (9 mg/L, point 11 of the OECD 301D guideline). If inhibition due to toxic effects is to be avoided, it is suggested in Annex II of OECD guideline 301 that the tested concentration should be <1/10 of EC_{50} in the activated sludge inhibition test. This implies a test concentration <0.23 mg/l for MIT. Therefore, toxic effects of the test substance on the inoculum at the actual test concentration of 10 mg/l cannot be excluded resulting in a less reliable test. In an additional non-key study, the degradation of MIT reached the maximum of 12 - 17 % after 29 days in a 36-day DOC die away ready biodegradability test. MIT did not exhibit clear inhibitory effects to activated sludge during the test.

5.1.2.3 Simulation tests

Simulation tests: aerobic biodegradation in a freshwater sediment system

Results of a water-sediment degradation study with MIT are summarised in Table 18. According to the TGD on Risk Assessment, the results from laboratory biodegradation studies should be recalculated to reflect an average EU outdoor temperature of 12 °C for the freshwater compartment. This recalculation was done with formula 25 of the TGD.

Table 18: Results from the freshwater-sediment degradation studies

Guide-line	Temp. [°C]	Initial conc. [mg/l]	Sediment Type	Rate Constant k [d^{-1}]	DT_{50} [d]	Reference
US EPA 162-4 Aerobic	25 ± 1°C	1	natural 1.1 % OM, 88 % sand, 6 % loam, 6 % clay	1.82 0.65 (12 ºC)	0.38 1.1 (12 ºC)	REYNOLDS J. L. (1994) (A7.1.2.2.2.a/01, Non-key study (Rohm and Haas))
US EPA 162-4 OECD draft 308 Aerobic	20 ± 1°C	1	Almshouse, natural 7.2 % OM, 31 % sand, 54 % silt, 15 % clay Cedar Hill,	1.51 0.80 (12 °C) 0.50	0.46[1] 0.87 (12 ºC) 1.4[2]	Schuck, H.(2002) (A7.1.2.2.2.a/02,Rohm and Haas)

			natural 2.3% OM, 58 % sand, 34% silt, 8 % clay	0.26	2.7 (12ºC)	
OECD 308	20 ± 2°C	0.5	Goorven, natural pH 6.1, 95 % sand, 1 % silt, 4 % clay Schoonrewoerdse wiel, natural pH 7.25, 35 % sand, 43 % silt, 22 % clay	0.555 (water) 0.542 (system) 0.0137 (water) 0.0131 (system)	1.25 (water)[3] 2.37(12 ºC) 1.28 (system) 2.43 (12 ºC) 2.11 (water)[3] 4.00 (12 ºC) 2.20 (system) 4.17 (12 ºC)	A7.1.2.2.2/01 (Thor GmbH)

[1] Calculated assuming first order kinetics, giving a good fit for the first 2 days of the study only
[2] Calculated assuming first order kinetics, giving a good fit for the first 7 days of the study only
[3] DT_{50} value relates to dissipation and not degradation

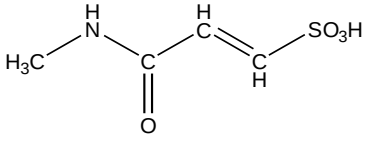
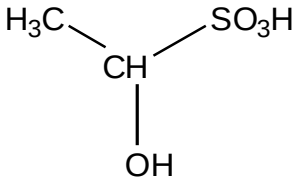
Rohm and Haas

MIT rapidly biodegrades in fresh water-sediment microcosms with a half-life varying from 0.38 to 1.4 days. Sediment bound residues reached maxima in the range of 59.4-67.76 % of applied radioactivity.

In the first study (A7.1.2.2.2.a/01), identification of metabolites was attempted but due to analytical difficulties definitive identification was not possible. Based on the production of $^{14}CO_2$ (available only with ring cleavage and oxidation) and the very polar chromatographic nature of the ^{14}C-activity, the metabolites have been characterized as N-methyl malonamic acid, N-methyl acetamide, N-methyl oxamic acid and malonamic acid. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation to N-(n-methyl) malonamic acid, N-(n-methyl) acetamide, N-methyl oxamic acid and/or malonamic acid. N-methyl malonamic acid, malonamic acid, and N-methyl acetamide have been shown to be readily biodegradable. About half of the radioactivity (~12% TAR) that could be extracted with 0.25N HCl from the sediment bound residue fraction was shown to consist of parent compound.

This study is only accepted as supplementary information. After addition of test substance to the water layer, the whole system was mixed. Mixing the whole system is not in line with the OECD 308 guideline. This procedure can be expected to affect the results of the study.

In the second study (A7.1.2.2.2.a/02), 2 metabolites were detected above 10 %, CO_2 and a metabolite (23.5 % of applied radioactivity) which was finally associated with 2 compounds: 2-(methylcarbamoyl) ethene sulfonic acid and 2-hydroxyethane sulfonic acid.

	
2-(methylcarbamoyl) ethene sulfonic acid	2-hydroxyethane sulfonic acid

The test substance was ^{14}C labelled in the 4 and 5 positions of the isothiazolone ring. Sampling was done directly after dosing and after 8 consequetive timepoints up to 30 days. The activity in the organic eluant increased until Day 2 in the Almshouse system (31.9% of the applied activity) and Day 7 in the Cedar Hill system (26.9%). Thereafter the activity decreased: at Day 30 it represented less than 6% of the applied activity. The ^{14}C-activity that was extracted from the sediment with methanol:KOH behaved similar to the organic eluant reaching a maximum in the Almshouse system of 6.7% of the applied activity on Day 2 and 4.4% on Day 7 in the Cedar Hill system. ^{14}C-activity detected in the KOH traps increased with time and comprised 27.9% of the applied activity on Day 30 in the Almshouse system and 18.4% in the Cedar Hill system. In the Almshouse system the nonextractable residues (PES) comprised 7.7% of the applied activity on Day 0.04 and increased to a maximum of 59.4% on Day 7. For the Cedar Hill system, the PES comprised 4.6% of the applied activity on Day 0.17 and reached a maximum of 61.5% on Day 30. The largest fraction of non-extractable activity remained in the unextractable inorganic humin fraction.

Thor GmbH

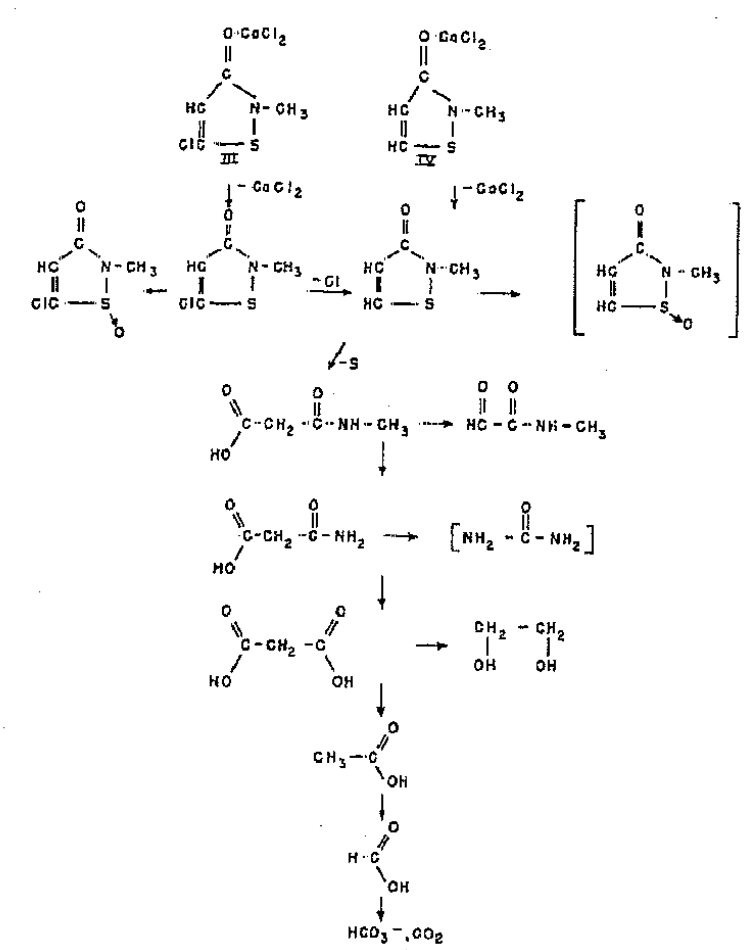
The test substance was ^{14}C labelled in the 4 and 5 positions of the isothiazolone ring. Sampling was done directly after dosing and after 8 consequetive timepoints up to 100-101 days. Upon addition of radiolabelled MIT to the water layer, applied radioactivity is rapidly partitioned between the aqueous and solid phase. Radioactivity in the water layer decreased to 17 and 10% AR within 39 and 58 days for the GV and the SW system, respectively. Mineralisation to carbon dioxide was a significant route of degradation as demonstrated by amounts of 42 and 24% AR after 100 and 101 days in the GV and SW system, respectively. Bound residues prior to Soxhlet extraction accounted for 42 and 47% AR after 39 and 58 days for GV and SW, respectively. MIT rapidly biodegrades in fresh water/sediment microcosms with a half-life varying from 1.28 to 2.20 days.

Based on TLC one major degradation product was formed in both aquatic systems, consisting apparently of two compounds or groups (M1 and M2), both of higher polarity than MIT based on HPLC analysis. None of those metabolites could be identified, because they were highly polar and of low molecular mass which prevented separation from the matrix or meaningful results by mass spectrometry. Concentrations of MIT in tests for biotic degradation need to be very low because of the biocidal effects of MIT at higher test concentrations. Therefore, any metabolites occur only at very low levels rendering identification work very difficult, especially of the metabolites that are small and highly polar. The proposed identity of metabolites based on LC-MS analysis cannot be considered fully confirmed as structures differed from the reference substances included in the study.

Published data are available on metabolic pathways for MIT and the structurally similar CIT in environmental matrices. Krzeminski (1975a, b) investigated degradation of [^{14}C] MIT and [^{14}C]CIT in various matrices including activated sludge and river water, using TLC, electrophoresis and GC-MS for characterisation and identification. The resulting common pathway for MIT and CIT is given in. First steps are CIT dechlorination and for both MIT and CIT the cleavage of the highly reactive N-S bond. That ring opening reaction is a mechanism well known for all isothiazolones (see e.g. Paulus W, 2005, Relationship between chemical structure and activity or mode of action of microbicides, page 13f).

The first identified metabolite was N-methylmalonamic acid for both MIT and CIT. Further oxidation and cleavage reactions lead to small, polar compounds such as malonamic acid, malonic acid, ethylene glycol, and finally to acetic acid, formic acid and hydrogen carbonate/carbon dioxide.

Figure 5.1.2c: Proposed pathway for biodegradation of MIT and CIT in the environment (Krzeminski 1975a, b)



Those findings confirm the formation of small, polar metabolites that also occurred in the studies on MIT described above.

Simulation tests: biodegradation in water

Results from aquatic biodegradation simulation tests are summarised in Table 19a. According to the TGD on Risk Assessment, the results from laboratory biodegradation studies should be recalculated to reflect an average EU outdoor temperature of 12 °C for the freshwater compartment and 9 °C for the marine environment. This recalculation was done with formula 25 of the TGD.

Table 19a: Results from the aquatic biodegradation studies

Guideline	MIT Conc. [µg/l]	Temp. [°C]	Half-Life [d]	Rate Constant k [d^{-1}]	Half-Life 9°C /12°C [d]	Rate Constant k 9°C / 12°C [d^{-1}]	Reference
OECD 309	22	20.05	1.38	0.501	3.34/2.63	0.207 / 0.264	A7.1.2.2.1/01

estuarine water	112	20.05	1.25	0.556	3.03/2.38	0.229 / 0.291	(Rohm and Haas)
OECD 309 freshwater	2	20±2	ND[1]	ND	-	-	A7.1.2.2.1-01 (Thor GmbH)
	97.5	20±2	<7[1]	ND	-	-	
OECD 309 seawater	1.5	15±2	ND	ND	NA	NA	Non-key study (Thor GmbH)
	87.5	15±2	3.6[2]	0.195	5.7	0.081	

[1] No reliable half-life can be calculated as no samples were taken between 0 and 7 days.
[2] Recalculated by RMS assuming first order kinetics, giving a good fit only for the first 7 days of the study

Rohm and Haas

Nutrient content of the estuarine water was determined as 6.6 ppm N, 7.7 ppm P and 8.6 ppm K. Total Organic Carbon in the estuarine water was determined as 20.0 ppm. Sampling was done directly after dosing and after 7 consequetive timepoints up to 144 hours. The ^{14}C-activity in the organic phase (methylene chloride) decreased with time going from 98.1% of the applied activity at 1 hour to 7 % after 144 hours for the 22 ppb dosing level and 93 % at time 0 to 6.2% after 144 hours for the 112 ppb dosing level. At the same time the activity in the aqueous phase (metabolites) increased with time from 0% of the applied dose after 1 hour to 43.3% for 22 ppb dosing level and 5 % at time 0 to 50.0 % after 72 hours for the 112 ppb dose. Biomass which consists of the emulsion phase during the partition and the residue during the concentration process increased as the incubation proceeded. At 144 hours it reached a maximum of 38.3-41.0 % of the applied dose. The biomass comprises polar metabolites that interact with surface water matrix such as dissolved organic matter and small particulates. During the course of the study very little ^{14}C-activity was evolved as volatiles. At both dosing concentrations total $^{14}CO_2$ detected in the KOH traps was less than 1% and no ^{14}C-activity was detected in the ethylene glycol traps.
MIT biodegraded very rapidly in the estuarine water studied. The half-lives were 1.38 days at 22 µg/l and 1.25 days at 112 µg/l, respectively.

The amount of ^{14}C activity in the methylene chloride fraction (fraction containing mostly parent) decreased with time while the activity in the aqueous fraction (metabolites mostly) increased. This indicates that MIT is being degraded, probably by ring cleavage and the formation of more polar alkyl metabolites. The major metabolite was identified by mass spectroscopy as N-methyl malonamic acid.

Thor GmbH

In freshwater MIT was found to degrade rapidly through biotic processes. Sampling was done directly after dosing and after 6 consequetive timepoints up to 56 days. After 56 days more than 95 % biodegradation of MIT had occurred. No conclusion can be drawn from this study regarding half-life of parent or formation of major metabolites as no samples were taken between 0 and 7 days. After 7 days only approximately 25 % of radioactivity was still present as MIT. The data are only considered as supportive for a rapid biodegradation of MIT in freshwater.
In a second study (non-key) the biodegradation of 14[C]-MIT in seawater was found to follow single first order kinetics only in the first week. Salinity of the estuarine water was determined as 30.9 promille. Total Organic Carbon in the estuarine water was determined as <3 ppm. The test substance was ^{14}C labelled in the 4 and 5 positions of the isothiazolone ring. Sampling was done directly after dosing and after 6 consequetive timepoints up to 56 days. The mass balance during the test ranged between 95 and 104 % for the 87.5 µg/l replicate, which was chosen for the HPLC measurements. The lowest concentration replicate exhibited recoveries of 112 - 123%, out of the acceptable range (90 - 110 %). HPLC analysis revealed seven unidentified metabolites (met 1-7) and three metabolite regions (Reg 1 - 3). The metabolites met-1, met-2 and met-3 and met-7 reached the highest

percentages of 19, 7, 6 and 5 %, respectively. They eluted after MIT and are considered to be less polar than MIT. Conversely, the other metabolites are considered to be more polar than MIT, as they had a shorter retention times.

Simulation test: sewage treatment plant

Results of an STP simulation test with MIT are summarised in Table 19b.

Table 19b: Results from aerobic sewage treatment simulation tests

Guideline / Test method	Test type	Temp. [°C]	Inoculum Type	Inoculum Conc.	Inoculum Adap-tation	MIT conc. [mg/l]	Half-Life [d]	Rate Constant k [d^{-1}]	Reference
Draft OECD 303A Activated Sludge Units	Aerobic biodegra-dability, flow-through	22±2	WWTP activated sludge	12 L/day equivalent to a retention time of 6 hours	no	0.100	1.69[1] 0.04[2] 0.03[3]	0.41 16.4 23.2	Oteyza, T., Gillings, E. and Roberts, G.C. (2007) (A7.1.2.1.1/01,Rohm and Haas)
TGD EEC 98/8 Draft OECD 303A	Aerobic biodegra-dability, static	20±2	WWTP activated sludge	2.5 g/l	no	0.25	0.02[4]	34.6	A7.1.2.1-01 Non-key study (Thor GmbH)

[1] Based on mineralization to CO_2
[2] Based on measured MIT concentration and assuming the entire radioactivity in suspended solids is MIT
[3] Based on measured MIT concentration and assuming none of the radioactivity in suspended solids is MIT
[4] Recalculated by RMS assuming first order kinetics, giving a good fit for the initial phase of degradation

Rohm and Haas

The test unit consisted of three main vessels: a mixing vessel, an aeration chamber and a settling vessel. Activated sewage was pumped into the mixing vessel at a rate of 12 l/day and 300 ml/day of the mixed liquor in the mixing vessel was transferred to a waste sludge flask. The hydraulic retention time in the mixing vessel was 6 hours and the sludge retention time, 10 days. The contents of the mixing vessel were transferred into an aeration vessel where the system was aerated with humidified air and then transferred to a settle vessel where the solids were allowed to settle and the supernatant transferred to a refrigerated effluent container. A pump transferred settled sludge solids back into the aeration vessel.
The unit was allowed to equilibrate for 20 days prior to dosing with ^{14}C-MIT. The dosing solution was transferred into the mixing vessel via a syringe pump. The dosing solution concentration was 100 mg/l and the delivery rate was 12 ml/day. The resulting concentration of ^{14}C-MIT in the mixing vessel was 100 µg/l. A steady state was obtained after 27 days of dosing and was maintained for 51 days.

The test substance was ^{14}C labelled in the 4 and 5 positions of the isothiazolone ring. In this simulation system, 63.76 %, 25.89 % and less than 2% of the applied activity was detected in the aqueous fractions, the solid fractions, and the volatiles, respectively. The effluent comprised a majority of the applied radioactivity with 60.6 % in the aqueous portion and 18.3 % in the solids. Parent comprised 11% of the applied activity in the effluent. While no metabolite was detected at greater than 10% of the applied activity, N-methyl malonamic acid, N-methyl acetamide, and malonamic acid were identified by LC-MS. In the waste sludge solids 6.6% of applied activity was found, but no extraction of waste sludge solids was performed. Therefore, it is not clear whether MIT was present in the waste sludge solids.

Thor GmbH

The test substance was 14C labelled in the 4 and 5 positions of the isothiazolone ring. Sampling was done 15 minutes after dosing and after 9 consequetive timepoints up to 168 hours. MIT degrades rapidly in the presence of activated sludge. A concentration of 0.25 mg/l was chosen in the study due to the potential for inhibitory effects at 1 mg/l, as observed in the preliminary tests. The results indicated that a fast mineralization occurred during the first hours after dosing, with a percentage of 14[C]O_2 formation of 7 % of total applied radioactivity (TAR) after 0.5 h incubation. The mineralization process slowed down after this first phase. After 2 days, no further biodegradation of MIT was observed and 18 % of the parent compound remained in the aqueous phase until the study end. After 7 days of incubation the amount of 14[C]O_2 was approximately 18 % TAR. The experiment was ended after 7 days of incubation due to the evaporation of the aqueous phase and the small amount of remaining sludge. The results of the study were assumed not to have been affected because a plateau phase had been reached after 7 days. Metabolites remained below 10 % of TAR during the course of the study. Metabolites were not identified, but presented a transient character.

Inhibitory effects of MIT on microbes start at concentrations less than 0.1 mg/l. Hence, inhibitory effects on activated sludge cannot be excluded at the test concentration of 0.25 mg/l. However, given the very short half-life for MIT in the test, such effects would exist for a very short time.

The study is a static test with only a single addition of radioactive-labeled test substance and not a continuously operated test system according to OECD 303A with a hydraulic retention time and a sludge age comparable to full-scale STPs. The results of the study can only be used as supplementary information for the degradation in STPs.

5.1.3 Summary and discussion of degradation

Abiotic degradation

Abiotic degradation of MIT in aqueous media occurs at a moderate rate and is significantly slower than aquatic biodegradation. Thus the primary route of dissipation in the environment is biological

In the troposphere, the calculated radical catalyzed degradation of MIT and its metabolites is very rapid resulting in half-life of 16.6 hours for the parent and 31.8 hours or less for metabolites.

Biodegradation

In ready biodegradation studies, **MIT was not found to be ready biodegradable.** Nevertheless, biological half-lives in the environment are very short, ranging from a couple of hours to a maximum of less than 3 days. Metabolism involves cleavage of isothiazolone ring and subsequent oxidation. The short half-life implies that the concentration of parent compound in the environment will be low. In sewage treatment simulation tests, metabolites remained below 10% of TAR during the course of the studies.

Metabolites in a first less reliable water-sediment study have been characterized but not definitively identified as N-methyl malonamic acid, N-methyl acetamide, N-methyl oxamic acid and malonamic acid. In a second water-sediment study two major metabolites have been tentatively identified as 2-(methylcarbamoyl) ethene sulfonic acid and 2-hydroxyethane sulfonic acid. In a third study MIT rapidly biodegraded in two water-sediment microcosms with a half-life of 1.28 and 2.20 days. One major degradation product was formed in both aquatic systems, consisting apparently of two

compounds or groups (M1 and M2), both of higher polarity than MIT. The proposed identity of metabolites cannot be considered definitive as structures differed from the reference substances included in this study.

In soil, the metabolic profile is expected to be similar. Two major metabolites have been tentatively identified as 2-methylcarbamoyl)-ethene sulfonic acid and 2-(methylcarbamoyl)-1-oxoethane sulfinic acid).

In all studies occurrence of metabolites was too transient to derive reliable values for the DT_{50}. RMS judged that more information on transformation products is not necessary because the substance is shown to be rapidly biodegradable. Moreover the applicants referred to relevant publications on the proposed degradation pathway of isothiazolones (see Fig. 5.1.2c). **The studies demonstrated that most of the metabolites are small polar compounds. In most cases they were rapidly biodegraded.**

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 20 summarizes the results of adsorption/desorption studies with MIT.

Table 20: Adsorption/desorption from soil and sediment

Guideline / Test Method	Soil Class	% a.i. adsorbed	K_a	K_{aoc}	K_d ((l/kg)	K_{doc} ((l/kg)	K_a/K_d ((l/kg)	Reference
US EPA 835-1110	Return activated sludge	2.6-51.1	20.11 - 56.82	ND	ND	ND	ND	Swales S. (2002) (A7.1.3/01, Rohm and Haas)
US EPA N163-1	Sandy loam	10.5	0.1	7.7	0.67	ND	0. 15	Gillings, E. (2006) (A7.1.3/03,Rohm and Haas)
	Clay loam	24.7	0.27	6.9	0.80	ND	0.34	
	Silty clay loam	16	0.14	6.7	0.91	ND	0.15	
	Sand	1.9	0.03	10	0.74	ND	0.041	
	Loam	46	1.07	6.4	0.96	ND	1.11	
OECD 121 (HPLC method)	NA	NA	NA	$2.88\ 10^{-25}$	NA	NA	NA	A7.1.3-01 (Thor GmbH)
OECD 106 & draft OECD (HPLC method)	NA	NA	NA	11.5	NA	NA	NA	Non-key study (Thor GmbH)

Rohm and Haas

When tested in an activated sludge adsorption test, the Freundlich sorption constant of MIT (K_f) was 6.12. The low value for the Freundlich sorption constant indicates that MIT is not extensively sorbed to activated sludge and likely to remain predominantely in the aqueous phase for the typical concentrations of sludge expected in a waste treatment plant. When tested in a soil adsorption test, MIT is adsorbed weakly to the examined soils and sediment. MIT is considered highly mobile.

An earlier study with three soils and one sediment (non-key study) showed that MIT is not stable under normal conditions because of microbial degradation. In the study listed above the soils were deactivated with gamma irradiation to avoid rapid biodegradation of MIT during the test. The

procedure to deactivate soils does not conform guideline OECD 106. The process of deactivation can influence the adsorption of MIT soil making the results of this test less reliable to estimate adsorption to soil under natural conditions. Additional QSAR results were obtained from the U.S. EPA's EPIWIN Suite version 3.1.2 (Meyland and Howard). The value obtained for MIT by the QSAR modelling was 3.4, which is in good agreement with the experimental values of 6.4-10.

Thor GmbH

The estimation of the adsorption coefficient (K_{OC}) on soil and sewage sludge was done with the HPLC method. The K_{OC} value of MIT was obtained by extrapolation, since no reference item with a shorter retention time than that of MIT was available. Based on the test results a character of very high mobility in soils was attributed to MIT (K_{OC} = $2.88 \cdot 10^{-25}$ l/kg). The value of $2.88 \cdot 10^{-25}$ is indicative as the value lies outside the range for reference substances.

In an earlier non-key study the degradation of MIT was too rapid to enable any adsorption/desorption evaluation according to the OECD Test Guideline 106. The adsorption coefficient of MIT could only be obtained by the HPLC method, through extrapolation of the calibration line. The estimated value for the K_{OC} of MIT is 11.5 l/kg.

5.2.1.1 Leaching in soil

When tested in a soil adsorption test, MIT is adsorbed weakly to the examined soils and sediment. MIT is considered highly mobile. However, due to its rapid biodegradation in soil, it is unlikely that parent mobility will be an environmental concern.

5.2.1.2 Conclusion on distribution

The available studies indicate a low adsorption potential of MIT. In sewage treatment plants and surface waters, MIT will be predominantly present in the water phase. The substance will not accumulate in sludge or sediments. MIT may have a potential for leaching in soil, but the rapid biodegradation of the substance in soil (half life < 0.5 day) indicates that the risk for groundwater can be considered very low.

5.2.2 Volatilisation

See paragraph 5.1.1. MIT is generally not expected to volatilise or partition to air to any relevant extent.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

MIT has a log Kow << 3 and its potential for bioaccumulation is negligible.

5.3.1.1 Bioaccumulation estimation

Aquatic bioconcentration

The experimental log K_{ow} values for MIT at pH 7 and 20 ºC was -0.32. The aquatic bioconcentration factor (BCF) for MIT has been estimated using QSAR according to the linear model generated by Veith *et al*. (1979)[2] (TGD, chapter 4, page 31) as this model is considered to be appropriate for substances with a log Kow < 6. The linear model is based on BCF data for fathead minnows (*Pimephales promelas*).

Log BCF_{fish} = 0.85 logKow - 0.70 (Veith *et al*, 1979)

The Log BCF_{fish} for MIT is estimated to be -0,972 and thus the BCF_{fish} is estimated to be 0,107.

Terrestrial bioconcentration

The BCF in earthworm was not experimentally determined. However, an equivalent approach to the aquatic system has been conducted using the equation as described by Jager (1998):

BCFearthworm = (0.84 + 0.012 KOW) / RHOearthworm where KOW MIT = 0.48 L/kg

RHOearthworm = 1 (default).

= (0.84 - 0.004) / 1 = 0.84

All calculations confirm the initial assumption of the negligible bioconcentration potential of MIT in biota. In addition MIT is rapidly biodegraded in the environment.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The risk of bioaccumulation or magnification of MIT is negligible.

5.4 Aquatic toxicity

A brief summary of the aquatic toxicity studies listed in the CAR for the three trophic levels fish, aquatic invertebrates and algae/aquatic plants are reported below. Only reliable and acceptable ecotoxicity tests from the CAR were used.

[2] Veith GD, Defoe DL and Bergstedt BV (1979). Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Board Can. **36**, 1040-1048.

Table 21: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
OECD 203 US EPA 72-1	LC_{50} 4.77 mg/L	96h, flow-through mean mesaured Rainbow trout *Oncorhynchus mykiss*	A7.4.1.1.a/01 (Rohm and Haas)
US EPA 72-1 US EPA OPPTS 850.1075	LC_{50} 5.71 mg/L	96h, semi-static mean measured Rainbow trout *Oncorhynchus mykiss*	A7.4.1.1.a/02 (Thor GmbH)
OECD 203 US EPA OPPTS 850.1075	LC_{50} 25.1 mg/L	96h, flow-through mean measured Sheepshead minnow *Cyprinodon variegatus*	A7.4.3.2b/01 (Rohm and Haas
OECD 202 US EPA FIFRA 72-2	EC_{50} 0.998 mg/L	48 h, flow-through mean measured *Daphnia magna*	A7.4.1.2.a/01 (Rohm and Haas)
US EPA FIFRA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300	EC_{50} 1.68 mg/L	48 h, semi-static measured *Daphnia magna*	A7.4.1.2/01 (Thor GmbH)
US EPA OPPTS 850.1035	LC_{50} 1.81 mg/L	96 h, flow-through mean measured Mysid shrimp *Americamysis bahia*	Hughes, C.(2004) (A7.4.1.2.b/01, Rohm and Haas)
OECD 201 US EPA FIFRA 122-2 EEC C.3	E_rC_{50} 0.103 mg/L (24 h) E_rC_{10} 0.062 mg/L (24 h)	120 h, static initial measured *Pseudokirchneriella subcapitata*	Hughes, C.(2004) (A7.4.1.3.b/01; Rohm and Haas)
US EPA FIFRA 123-2 US EPA OPPTS 850.5400	ErC_{50} 0.114 mg/L (24 h) E_rC_{10} 0.024 mg/L (24 h)	96 h, static initial measured *Pseudokirchneriella subcapitata*	(A7.4.1.3-01, Thor GmbH)
US EPA FIFRA 123-2	E_rC_{50} 0.0695 mg/L (24 h) E_rC_{10} 0.044 mg/L (24 h)	120 h, static initial measured *Skeletonema costatum*	Hughes, C.(2004) (A7.4.1.3.b/01, Rohm and Haas)
OECD 210 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600	NOEC 2.38 mg/L (growth,wet weight)	98 days, flow-through mean measured Rainbow trout *Oncorhynchus mykiss*	A7.4.3.2a/01 (Rohm and Haas)
OECD 210 US EPA OPPTS 850.1400	NOEC 2.1 mg/L (survival)	33 days, flow-through mean measured Fathead minnow *Pimephales promelas*	A7.4.3.2/01 (Thor GmbH)
OECD 211 US EPA OPPTS 850.1300	NOEC 0.0442 mg/L (dry weight)	21 days, flow-through measured *Daphnia magna*	Hicks SL (2004) (A7.4.3.4/01,Roh m and Haas)
OECD 211 US EPA OPPTS 850.1300	NOEC 0.55 mg/L (dry weight)	21 days, flow-through measured *Daphnia magna*	A7.4.3.4/01 (Thor GmbH)
OECD 218 Spiked sediment test	NOEC 13.0 mg/kg dw sed. (rate of development)	28 days, static nominal *Chironomus riparius*	Aufderheide J. (2006) (A7.4.3.5.1a/01,R ohm and Haas)
Draft OECD Sediment-Water *Lumbriculus* Toxicity test Using Spiked Sediment Guideline, September 2006	NOEC 25 mg/kg dw sed. (survival)	28 days, static nominal *Lumbriculus* variegates (Oligochaete)	Thomas S.T., Krueger, H.O., Kendall, T.Z. and Nixon, W.B. (20007) (A7.4.3.5.1a/02,R ohm and Haas)
US EPA OPPTS 850.1735 a.i.TM E 1706-00	NOEC 13.0 mg/kg dw sed.	28 days, static nominal *Hyallela azteca* (amphipod)	Thomas S.T., Krueger, H.O., Kendall, T.Z. and Nixon, W.B. (2008) (A7.4.3.5.1.a/03,R ohm and Haas)

5.4.1.1 Short-term toxicity to fish

Rohm and Haas
An acute toxicity tests was performed with rainbow trout (*Oncorhynchus mykiss*). The 96 h LC_{50} for *O. mykiss* of 4.77 mg a.i./l is based on mean measured concentrations. The 96 h LC_{50} for sheepshead minnow (*Cyprinodon variegatus*) was 25.1 mg/l, based on mean measured concentrations.

Thor GmhH
An acute toxicity tests was performed with rainbow trout (*Oncorhynchus mykiss*). The study showed a steep dose-response curve. Partial mortality has only been observed at one concentration, whereas complete mortality has been reported at the next tested concentrations.

5.4.1.2 Long-term toxicity to fish

Rohm and Haas
A chronic flow-through early life stage toxicity tests was also performed with rainbow trout (*Oncorhynchus mykiss*). The most sensitive endpoint was growth (total length and wet weight). The NOEC in this study is 2.38 mg a.i./L.

Thor GmhH
In the chronic flow-through early life stage toxicity test with fathead minnow (*Pimephales promelas*), no sublethal effects were recorded in the test concentrations without significant effects on survival. The lowest chronic value is the NOEC for survival of *P. promelas* of 2.1 mg a.s./l.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Rohm and Haas
The EC_{50} of 0.850 mg/L for *Daphnia magna* proposed in the original study report was considered not reliable. In the test system with a higher mean measured concentration of 0.865 mg/L only 7 out of 20 Daphnia were recorded as dead or immobile. The result was not consistent with the dose-response curve. The graphical representation illustrated that the EC_{50} should be higher than 0.865 mg/L. The moving average method was used in the study to calculate 48 hours EC_{50} and LC_{50} values. OECD 203 recommends using a probit method. RMS recalculated the EC_{50} with the trimmed Spearmann-Karber method based on pooled results of the replicas. The resulting 48 h EC_{50} was 0.998 mg a.i./l based on mean measured concentrations. Mean measured concentrations ranged from 81 to 92 % of nominal concentrations.

A flow-through test on mysid shrimp (*Americamysis bahia*) gave an acute toxicity value for marine invertebrates exposed to MIT. The 96 h LC_{50}, based on mean measured concentrations was 1.81 mg a.i./l.

Thor GmbH
Due to a steep dose-response curve, there was just a single test concentration at which part of the Daphnia died or immobilized. Concentrations were below 80% of initial at the end of the test at concentrations 0.58 and 1.0 mg/L, with 77 and 74%, respectively. However,

concentrations were within the 80-120% of the nominal concentrations as required by the OECD-Guideline. The measured concentrations ranged from 98 to 113% of the nominal concentrations in the fresh medium and from 74 to 94% of the initial concentration in the old medium.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Rohm and Haas

The lowest chronic value from a flow-through chronic toxicity test with *D. magna* was the 21-day $NOEC_{growth}$ of 0.0442 mg a.i./l, based on significant effects on dry weight at the next concentration level of 0.0889 mg a.i. /L. Hower, there was no clear dose–response as no significant effect was seen at 0.183 mg a.i./L and no significant effects on reproductive endpoints were found up to the highest tested concentration level of 0.359 mg a.i./L. It should be noted that growth is an optional test parameter according to guideline OECD 211.

Thor GmbH

Effects on growth were not tested in the flow-through chronic toxicity test with *D. magna*. The measured concentrations ranged from 100 to 112% of the nominal concentrations.
The study resulted in the lowest NOEC for survival of the first generation (0.55 mg a.s./l).

5.4.3 Algae and aquatic plants

The study results are presented in details in Annex 2 of the CLH report.

Rohm and Haas

A MIT toxicity test with the freshwater alga *Pseudokierchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) is summarised in Table 21.
The concentration of test substance was not maintained at >80 % of nominal concentrations in both tests, due to fast biodegradation in the presence of algae. At the lower test concentrations, MIT was completely degraded within 120 hours and not found above the limit of detection at the end of the test. There is a clear concentration dependency in the degradation of MIT. Degradation is relatively slower in 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 hours of the test. Calculated values for 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 hours. 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 201 (2006) and was not applicable at the time the study was performed (1997). It was however agreed that this study is acceptable and that as suggested by RMS the 24h-E_rC_{10} based on initial measured concentrations is the appropriate endpoint to be considered in the PNEC derivation. Analogous the 24h-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 standard 72h NOEC is proposed as the endpoint should reflect effects over longer test duration rather than effects in the early phase of the exposure.

The toxicity of MIT towards the marine alga *Skeletonema costatum* was also tested. The results are summarised in Table 21.

The test substance concentrations were measured at 0 and 120 hours. Except for the media with the two highest initial test concentrations, all concentrations were below detection limit at 120 hours. Because of the rapid removal of MIT from the test system, and the lack of MIT measurements between 0 and 120 hours, the 96 hour NOErC of 0.0725 mg a.i./l (based on initial measured concentrations) cannot be considered a reliable endpoint. It was however agreed that this study is acceptable and that as suggested by RMS the 24h-E_rC_{10} based on initial measured concentrations is the appropriate endpoint to be considered in the PNEC derivation. Analogous the 24h-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 standard 72h NOEC is proposed as the endpoint should reflect effects over longer test duration rather than effects in the early phase of the exposure.

Thor GmbH

A MIT toxicity tests with the freshwater alga *Pseudokierchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) is available (see Table 21).

The concentration of test substance was not maintained at >80% of nominal concentrations in the tests, due to fast biodegradation in the presence of algae. At the lower test concentrations, MIT was completely degraded within 96 hours and not found above the limit of detection at the end of the test. There was a clear concentration dependency in the degradation of MIT. Biodegradation is relatively slower in 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 algae study shows, 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 with time. E_rC_{50} values calculated following independent statistical analysis increased from 0.114 mg/l over the first 24 hours to 0.118 µg/l over 48 hours and 0.160 µg/l over 72 hours.

The concentration dependency in the degradation of MIT in both studies can be attributed to the role of algae in the degradation of MIT. MIT is rapidly taken up by the algae, and inhibits enzymes by the binding to the thiol-groups of the proteins. A consequence of this binding is cleaving of the isothiazolone ring and further degradation. This means that the inhibitory effect on algae also will result in a degradation of MIT by algae. At higher test concentrations toxic to algae, growth of algae is inhibited which in turn slows down the degradation of MIT by algae. The mode of action of MIT implies that the sensitivity of the test is affected by the cell density.

Obviously, 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 hours NOEC based on nominal concentrations can not 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.

The 24 hour NOE_rC or E_rC_{10} based on initial measured concentrations should in the risk assessment be used as endpoint from these studies. Using the 24 hours value is not a standard approach, as the general recommendations of the OECD 201 are to use the 72 hours interval with a possibility to reduce the duration to 48 hours. However, the case of MIT is very similar to DCOIT. MIT has a unique mode of action in algae. Like DCOIT, MIT is a fast acting biocide and toxicity is stoichiometric and closely associated with degradation. Analogous the 24h-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 standard 72h NOEC is proposed as the endpoint should reflect effects over longer test duration rather than effects in the early phase of the exposure.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Toxicity to sediment dwelling organisms

Rohm and Haas

Chronic sediment toxicity test were conducted with larvae of *Chironomus riparius* and the amphipod *Hyalella azteca*. Most sensitive endpoint of the *Chironomus* test was the NOEC of 13.0 mg/kg dry sediment for developmental rate. The NOEC derived from the test with oligochaete *Lumbricius variegates* was 25 mg/kg dry sediment, based on adult survival. Endpoints are to be treated with caution, because actual exposure to the test substance could have been very short due to rapid biodegradation of MIT in the test systems. The NOEC derived from the test with *Hyalella azteca* was 13 mg/kg dry sediment, based on adult survival.

5.5 Comparison with CLP classification criteria for environmental hazards (sections 5.1 – 5.4)

CLP- Acute aquatic hazards

The lowest available $L(E)C_{50}$ value relevant for classification of MIT is the 24 h E_rC_{50} of **0.0695 mg** a.i./l obtained for the marine alga species *Skeletonema costatum*. Based on this lowest $L(E)C_{50}$ value MIT fulfils the criteria $L(E)C_{50} \leq 1$ mg/l for classification as **Acute aquatic Category 1**, **H400** (Very toxic to aquatic life) with an **M-factor of 10** due to the 24 h E_rC_{50} in the range $0.1 < L(E)C_{50} \geq 0.01$.

CLP - Aquatic chronic hazards

The lowest $NOEC/EC_{10}$ is the 24 h E_rC_{10} of 0.024 mg a.i./l obtained for the freshwater alga species *Pseudokierchneriella subcapitata*. Available NOEC values for fish and Daphnia are higher. The lowest endpoint value for algae fulfils the criteria $NOEC/EC_x \leq$ 0.1 mg/l. Being not rapidly degradable, MIT therefore in principle fulfils criteria for classification as **Aquatic Chronic Category 1**, **H410 (**Very toxic to aquatic organisms with long lasting effects) with an M-factor of 1 due to the 24 h E_rC_{10}in the range $0,1 < NOEC/EC_x \geq 0,01$. But MIT is a fast acting biocide and toxicity is stoichiometric and closely associated with degradation. Given the specific mechanism for the toxicity of MIT to algae it can be foreseen that the parent in fact has no long-lasting effects on algae if the number of algae is not limited.

MIT is considered not readily degradable, but simulation tests show rapid primary biodegradation of MIT in the environment to metabolites of which most are demonstrated or expected to be less toxic than MIT. However, not all metabolites

formed at >10% have been successfully identified. According to Regulation (EC) No 1272/2008, primary biodegradation data can be used to justify a non-chronic classification of the parent substance if the degradation products shall not be classified as hazardous to the aquatic environment (one of the Acute or Chronic Categories). **Hence, definitive identification of all metabolites reaching >10% in aquatic biodegradation studies is required to justify a non-chronic classification of MIT.**

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

In accordance with the provisons of CLP Regulation (EC) No 12272/2008 MIT shall be assigned with pictogram **GHS09,** with signal word "Danger" and with the following hazard statements: **H410 (**Very toxic to aquatic organisms with long lasting effects).

An **acute M factor of 10 will** be applied, due to the 24 hours E_rC_{50} of 0.0695 mg/l from the *Skeletonema costatum* study. An **chronic M factor of 1** will be applied, due to the 24 h E_rC_{10} of 0.024 mg a.i./l from the *Pseudokierchneriella subcapitata* study.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

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

Degradation

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

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

Three ready biodegradation studies are available showing that MIT is not readily biodegradable. In the first one, carried out according to OECD TG 301B (Modified Sturm

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

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

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

Bioaccumulation

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

Aquatic toxicity

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

Summary of relevant information on aquatic toxicity.

Method	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
OECD TG 203 US EPA 72-1 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	96-h LC_{50}	4.77	A7.4.1.1.a/01 (Rohm and Haas)
US EPA 72-1 US EPA OPPTS 850.1075 Freshwater	*Oncorhynchus mykiss*	Semi-Static mm	96-h LC_{50}	5.71	A7.4.1.1.a/02 (Thor)
OECD 203 US EPA OPPTS 850.1075 Marine water	*Cyprinodon variegates*	Flow-through mm	96-h LC_{50}	25.1	A7.4.3.2.b/01 (Rohm and Haas)
Long-term toxicity to fish					
OECD TG 210 US EPA OPPTS 850.1075 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	98-d NOEC (based on growth, wet weight)	2.38	A7.4.3.2.a/01 (Rohm and Haas)
OECD TG 210 US EPA OPPTS 850.1075 Freshwater	*Pimephales promelas*	Flow-through mm	33-d NOEC (survival)	2.1	A7.4.3.2/01 (Thor)
Short-term toxicity to aquatic invertebrates					
OECD TG 202 US EPA 72-2 Freshwater	*Daphnia magna*	Flow-through mm	48-h EC_{50}	0.998	A7.4.1.2.a/01 (Rohm and Haas)
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Semi-Static m	48-h EC_{50}	1.68	A7.4.1.2/01 (Thor)
US EPA OPPTS 850.1035 Marine water	*Americamysis bahia*	Flow-through mm	96-h LC_{50}	1.81	A7.4.1.2.b/01 (Rohm and Haas)
Long-term toxicity to aquatic invertebrates					
OECD TG 211 US EPA OPPTS 850.1300 Freshwater	*Daphnia magna*	Flow-through m	21-d NOEC (based on dry weight)	0.0442	A7.4.3.4/01 (Rohm and Haas)
OECD TG 211 US EPA OPPTS 850.1300 Freshwater	*Daphnia magna*	Flow-through m	21-d NOEC (based on dry weight)	0.55	A7.4.3.4/01 (Thor)
Toxicity to algae					
OECD TG 201 US EPA FIFRA 122-2 EEC.3 Freshwater	*Pseudokirchneriella subcapitata*	120h Static imc	24-h E_rC_{50} 24-h E_rC_{10}	0.102 0.062	A7.4.1.3.b/01 (Rohm and Haas)
US EPA FIFRA 123-2 US EPA OPPTS 850.5400 Freshwater	*Pseudokirchneriella subcapitata*	96h Static imc	24-h E_rC_{50} **24-h E_rC_{10}**	0.114 **0.024**	A7.4.1.3-01 (Thor)
US EPA FIFRA 123-2	*Skeletonema costatum*	120h Static imc	**24-h E_rC_{50}**	**0.0695**	A7.4.1.3.b/01 (Rohm and

Marine water			24-h E_rC_{10}	0.044	Haas)

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

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

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

Regarding toxicity to algae, the DS provided two toxicity studies on *Pseudokirchneriella subcapitata* and a toxicity study on the saltwater diatom *Skeletonema costatum*. All the studies are static tests and the derived endpoints were based on initial measured concentrations. The concentration of the test substance was not maintained at >80% of nominal concentrations, due to fast biodegradation of MIT in the presence of algae, and the exponential increase in cell density in the controls was not maintained after 72 h. This can be attributed to the peculiar behaviour of the substance in the presence of algae by means that the degradation of MIT depends on the algal concentration (*i.e.* the concentration dependency can be attributed to the role of algae in the degradation of MIT). MIT is rapidly taken up by the algae, and inhibits enzymes by binding to the thiol-groups of the proteins. A consequence of this binding is cleaving of the isothiazolone ring and further degradation. This means that the inhibitory effect on algae will also result in a degradation of MIT by algae. At higher test concentrations toxic to algae, growth of algae is inhibited which in turn slows down the degradation of MIT by algae. The mode of action of MIT implies that the sensitivity of the test is affected by the cell density. Therefore, the removal of MIT from the test system is rapid and a NOEC based on geometric mean concentration does not take into account the interaction between algal density and biodegradation of MIT. For this reason, the 24 hour E_rC_{10} based on initial measured concentrations was used as endpoint.

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

Summary of relevant information on aquatic toxicity of MIT metabolites

Method	**Test organism**	**Conditions/Metabolite**	**Endpoint**	**Toxicity values in mg a.s./L**	**Reference**
Short-term toxicity to fish					
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static nom N-methyl malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/01 (Rohm and Haas)
OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm N-methyl acetamide	96-h LC_{50}	>694	A7.4.1.1.c/02 (Rohm and Haas)

OECD TG 203 US EPA OPPTS 850.1400 US EPA FIFRA 72-4 US EPA TSCA 797.1600 Freshwater	*Oncorhynchus mykiss*	Static mm malonamic acid	96-h LC_{50}	>1000	A7.4.1.1.c/01 (Rohm and Haas)
Short-term toxicity to aquatic invertebrates					
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static nom N-methyl malonamic acid	48-h EC_{50}	> 1000	A7.4.1.2.c/01 (Rohm and Haas)
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static mm N-methyl acetamide	48-h EC_{50}	> 863	A7.4.1.2.c/02 (Thor)
US EPA 72-2 US EPA OPPTS 850.1010 US EPA 40 CFR 797.1300 Freshwater	*Daphnia magna*	Static nom malonamic acid	48-h EC_{50}	> 1000	A7.4.1.2.c/03 (Rohm and Haas)
Toxicity to algae					
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96h-Static imc N-methyl malonamic acid	72-h E_rC_{50} 96-h E_rC_{50} 72-h NOE_rC 96-h NOE_rC	97 128 36 36	A7.4.1.3c/01 (Rohm and Haas)
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96h-Static imc N-methyl acetamide	72-h E_rC_{50} 96-h E_rC_{50} 72-h NOE_rC 96-h NOE_rC	5.8 5.7 0.51 0.51	A7.4.1.3c/02 (Rohm and Haas)
OECD TG 201 US EPA OPPTS 850.5400 US EPA TSCA 797.1050 US EPA FIFRA 122-2 and 123-2	*Pseudokirchneriella subcapitata*	96h-Static imc Malonamic acid	72-h ErC50 96-h ErC50 72-h NOErC 96-h NOErC	> 1080 > 1080 1080 519	A7.4.1.3c/03 (Rohm and Haas)
mm – mean measured concentration nom – nominal concentration imc – initial measured concentration					

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

Comments received during public consultation

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

Regarding aquatic toxicity, one MSCA supported the use of the 24h-ErC_{10} endpoint on algae for classification and proposed some modifications to harmonise some sections of the CLH report, which were agreed by the DS. According to another MSCA, it was not clear if the algal endpoint reflected the validity criteria of exponential growth in controls at 48h and therefore proposed that the classification should be based on endpoints which reflect the usual period of exponential growth (72h or 96h). The DS replied that the exponential growth in the control

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

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

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

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

Additional key elements

The ecotoxicity studies used by the DS in order to classify the substance, both for short (Rohm and Haas) and long-term hazards (Thor) were based on 24h algae tests. The OECD TG 201 and the ECHA guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b foresee the possibility to adopt a shortened test period (48 h) with respect to the usual duration of 72h or 96h, but the 24h length for this test is not mentioned in the guidance. Also the Guidance on the Application of the CLP Criteria foresees the possibility to adopt a shortened test period: *"For classification and labelling purposes […] differing test duration could be used if no other acceptable data are available".*

In addition, the validity criteria for the control performance (exponential control growth greater than a factor of 16) seem not to be fulfilled in the first 24h, in both acute and chronic tests. Nevertheless, the criteria for the specific growth rate > 0.92 d^{-1} were met.

Moreover, it is important to highlight that MIT is a fast-acting biocide and toxicity is closely associated with degradation. Evaluation of the algae study showed that the effect on the growth pattern is mainly related to the effect in the early phase of exposure due to the fact that concentrations of MIT are declining with time. The strongest adverse effect is shown at 24h and at 48h the effect is already clearly lower, as reported in the test evaluation performed by the RMS in the biocidal framework assessment.

In the evaluation of the ecotoxicity of the substance C(M)IT/MIT (CAS n. 55965-84-9), which is also an isothiazolinone and has a similar mode of action on algae as MIT, the endpoint chosen for the classification was a 48h E_rC50 and NOE_rC for algae. The difference for MIT

behaviour can be explained only on the basis of statistical analysis of the test results, as reported in the evaluation of the RMS.
The results of the key studies are based on initial measured concentration, notwithstanding the use of mean measured concentrations, like in the key study of C(M)IT/MIT, would be more appropriate due to the behaviour of the substance (degradation in presence of algae). The use of imc (initial measured concentration) could underestimate the ecotoxicity of the substance resulting in lower M-factors, but the measured concentrations at 24h were not available.

In a weight of evidence approach, the long-term study on *Daphnia*, which provided a NOEC value of 0.0442 mg/L of the same order of magnitude of the algae test (0.024 mg/L) could support the proposed classification for long-term hazards. It is necessary to take into account that the NOEC for *Daphnia* was not based on reproduction effects but on growth (dry weight), which is only an optional test parameter according to OECD TG 211. It should be noted that the CLH report did not provide detailed information for this test.

Assessment and comparison with the classification criteria

Degradation

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

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

Bioaccumulation

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

Aquatic toxicity

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

Conclusion on the classification

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

6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for evaluation of 2 -methylisothiazol-3(2*H*)-one (MIT) according to Regulation (EU) No. 528/2012. The summaries included in this proposal are partly copied from the CAR. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of MIT. More details shall be found in the CAR then.

7 REFERENCES

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List of studies (Rohm and Haas):

Section No / Reference No	Author(s)	Year	Title. Source (if different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Y/N)	Owner
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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-METHYLISOTHIAZOL-3(2H)-ONE (ISO)

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-METHYLISOTHIAZOL-3(2H)-ONE (ISO)

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Section No / Reference No	**Author(s)**	**Year**	**Title. Source (if different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Y/N)**	**Owner**
			Unpublished.		
A7.4.3.4/01	Hicks SL	2004	2-Methyl-4-isothiazolin-3-one: Chronic toxicity test with the water flea, *Daphnia magna*, conducted under flow-through conditions. ABC Laboratories Study N° 48836, Rohm and Haas Report N° 04RC-0024, November 8, 2004, Unpublished.	**Y**	Rohm and Haas
A7.4.3.5.1.a/01	Aufderheide J.	2006	2-methyl-4-isothiazolin-3-one: Chronic toxicity in whole sediment to freshwater midge *Chironomus riparius.* ABC Laboratories Study N° 49009, Rohm and Haas Report N° 04RC-055 (January 25, 2006), Unpublished.	**Y**	Rohm and Haas
A7.4.3.5.1.a/02	Thomas S.T., Krueger, H.O., Kendall, T.Z. and Nixon, W.B.	2007	2-methyl-4-isothiazolin-3-one: A sediment-water *Lumbriculus* toxicity test using spiked sediment, Wildlife International Ltd Project No 129A-131, Rohm and Haas report No 06RC-227 (July 19, 2007), Unpublished	**Y**	Rohm and Haas
A7.4.3.5.1.a/03	Thomas S.T., Krueger, H.O., Kendall, T.Z. and Nixon, W.B.	2008	2-methyl-4-isothiazolin-3-one: A prolonged sediment toxicity test with *Hyalella azteca* using spiked sediment, Wildlife International Ltd Project No 129A-131, Rohm and Haas report No 06RC-227 (July 19, 2007), Unpublished	**Y**	Rohm and Haas

List of studies (Thor GmbH):

Section No / Reference No	**Author(s)**	**Year**	**Title** **Source (where different from company)** **Company** **Report No.** **GLP (where relevant)** **(Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
III-A 6.1.1-01	[redacted]	2000	Acute Oral Toxicity Study of Acticide SR 3267 in Rat; [redacted]; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.1.2-01	[redacted]	2000	Acute Dermal Toxicity Study of Acticide SR 3267 in Rat - Limit Test; [redacted]; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.1.3-01	[redacted]	2000	Acute Inhalation Toxicity Study of Test Item Acticide SR 3267 in Rats; [redacted] [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.1.4-01/1	[redacted]	2000	Acute Dermal Irritation/Corrosion Test of Acticide SR 3267 in Rabbits; [redacted] [redacted] Unpublished	Y	Thor GmbH
III-A 6.1.5-01/1	[redacted]	2000	Sensitization Study of Acticide SR 3267 in Guinea Pig Maximization Test According to Magnusson and Kligman; [redacted] [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.2-01	[redacted]	1998	(14C)-CIT and (14C)-MIT: Absorption, distribution, metabolism and excretion following oral administration to the rat; [redacted] [redacted] GLP; Unpublished	Y	Thor GmbH

Section No / Reference No	Author(s)	Year	Title Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/No)	Owner
III-A 6.2-02	[redacted]	2000	(14C)-CIT and (14C)-MIT: Characterisation of metabolites following oral administration to the rat; Covance Laboratories GmbH; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.2-03	[redacted]	1982	^{14}C-Kathon 886 disposition after percutaneous application to male rats; Toxicology department, Rohm and Haas Company [redacted] Unpublished	N	Thor GmbH
III-A 6.6.2-1	[redacted]	2002	In vitro Mammalian Chromosome Aberration Test of ACTICIDE M 50 with Human Lymphocytes; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.6.3/1	[redacted]	2000	Mutagenic Evaluation of Test Item Acticide SR 3267 in CHO/HPRT Assay; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.6.4-1	[redacted]	2000	Mutagenic Effect of Test Item ACTICIDE SR 3267 by Micronucleus Test; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.6.5/1	[redacted]	1994	Study to Evaluate the Potential of ACTICIDE 14 to Induce Unscheduled DNA Synthesis in Rat Liver using an in vivo/in vitro Procedure; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 6.8.1-02	[redacted]	2000	Teratogenicity study of test item ACTICIDE SR 3267 in rats; [redacted] GLP;	Y	Thor GmbH

Section No / Reference No	Author(s)	Year	Title Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/No)	Owner
			Unpublished		
III-A 6.8.2	[redacted]	2003	A Two-Generation reproductive development toxicity study of 2-Methyl-4-isothiazolin-3-one administered via drinking water in rats; [redacted] [redacted]; GLP; Unpublished	Y	Thor GmbH
III-A 7.1.1.2.1-01	??				
III-A 7.1.2.2.1-01	[redacted]	2007	The determination of degradation of 2_Methyl-2H-isothiazol-3-one (MIT, CAS *2682-20-4) in seawater (OECD guideline 309); [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 7.4.1.2-01	[redacted]	1999	ACTICIDE SR 3267: Aquatic Invertebrate Acute Toxicity Test (48 h), Freshwater Daphnids: Daphnia magna STRAUS; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 7.4.1.3-01	[redacted]	1999	ACTICIDE SR 3267: Algal Toxicity, Pseudokirchneriella subcapitata, 96 h; [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 7.4.3.2	[redacted]	2006	2-Methyl-2H-isothiazol-3-one (MIT, Applied as Aqueous Formulation ACTICIDE® M 20): An Early Life-Stage Toxicity Test with the Fathead Minnow (Pimephales promelas); [redacted] GLP; Unpublished	Y	Thor GmbH
III-A 7.4.3.4	[redacted]	2006	2-Methyl-2H-isothiazol-3-one (MIT; Applied as Aqueous Formulation ACTICIDE® M 20): A Flow-Through Life-Cycle Toxicity Test with the Cladoceran	Y	Thor GmbH

Section No / Reference No	Author(s)	Year	Title Source (where different from company) Company Report No. GLP (where relevant) (Un)Published	Data Protection Claimed (Yes/No)	Owner
			(Daphnia magna); [redacted] [redacted] GLP; Unpublished		

List of publications on sensitisation:

Ackermann L, Aalto-Korte K, Alanko K, Hasan T, Jolanki R, Lammintausta K, Lauerma A, Laukkanen A, Liippo J, Riekki R, Vuorela AM, Rantanen T. (2011). Contact sensitisation to methylisothiazolinone in Finland--a multicentre study. Contact Dermatitis 64 (1): 49-53

Alwan W, White IR, Banerjee P (2014). Presumed airborne contact allergy to methylisothiazolinone causing acute severe facial dermatitis and respiratory difficulty. Contact Dermatitis 70(5): 320-1

Kaae J, Menne T, Thyssen JP (2012). Presumed primary contact sensitization to methylisothiazolinone from paint: a chemical that became airborne.Contact Dermatitis 66(6):341-2.

Lundov MD, Mosebech H, Thyssen JP, Menne T, Zachariae C (2011). Two cases of airborne allergic contact dermatitis caused by methylisothiazolinone in paint, Contact Dermatitits 65(3): 176-9.

Lundov MD[1], Opstrup MS, Johansen JD (2013). Methylisothiazolinone contact allergy--growing epidemic. Contact Dermatitis 69(5):271-5

Lundov MD, Thyssen JP, Zachariae C, Johansen JD (2010). Prevalence and cause of methylisothiazolinone contact allergy. Contact Dermatitis 63(3): 164-7

Lundov MD, Zachariae C, Johansen JD (2011). Methylisothiazolinone contact allergy and dose–response relationships. Contact Dermatitis 64(6): 330–6

Mose AP, Lundov MD, Zachariae C, Menné T, Veien NK, Laurberg G, Kaaber K, Avnstorp C, Andersen KE, Paulsen E, Gotthard Mortz C, Sommerlund M,Danielsen A, Thormann J, Kristensen O, Kristensen B, Andersen BL, Vissing S, Nielsen NH, Johansen JD (2012). Occupational contact dermatitis in painters: an analysis of patch test data from the Danish Contact Dermatitis Group.Contact Dermatitis 67(5): 293-7

Urwin M, Wilkinson L (2013). Methylchloroisothiazolinone and methylisothiazolinone contact allergy: a new ‘epidemic’. Contact Dermatitis 68(4): 250-6

Uter W, Geier J, Bauer A, Schnuch A (2013). Risk factors associated with methylisothiazolinone contact sensitisation. Contact Dermatitis 69(4), 231-8

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Makris SL, Solomon MH, Clark r, Shiota K, Barbellion S, Buschmann J, Ema M, Fukiwara M, Grote K, Hazalden KP, Hew KW, Horimoto M, Ooshima Y, Parkinson M and Wise LD (2009). Terminology of developmental abnormalities in common laboratory mammals (version 2). Reproductive Toxicology, 28, 371-434.

Vauhkala A-R, Pesonen M, Suomela S, Kuuliala O, Suuronen K and Aalto-Korte K (2015). Occupational contact allergy to methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone. Contact Dermatitis, 73, 150-156.

Yazar K, Lundov MD, Faurschou A, Matura M, Boman A, Johansen JD and Liden C (2015). Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open-application study. British J of Dermatology, 173, 115-22.

Warburton, K.L. and Wilkinson, M. (2015). Contact allergy to methylisothiazolinone: has there been any change? Experience of a UK centre. Contact Dermatitis 72, 398–421.

SCCS (2015): Opinion on Methylisothiazolinone (P94) Submission II (Sensitisation only), SCCS 1557/15. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_178.pdf

8 ANNEX

Annex 1: final Draft Competent Authority Report (CAR) - Document II-A - Study Summaries – MIT (September 2014)

Annex 2: **SI-consultation (31 January 2013) Endpoints from algae studies with MIT and aquatic PNEC**

Background

The endpoints from algae studies with MIT and derivation of the aquatic PNEC was discussed in TMIV 2012. The main points as summarized in the TMIV2013 draft minutes, version1:

- Because of the specific mode of action of the *isothiazolinones* (rapidly reacting), algal cell density at the start of the exposure has a large influence on the outcome of the test. Low initial cell density increases the sensitivity in the test, and vice versa. Accordingly, the outcomes of different tests can only be compared if initial algal cell density is similar. For DCOIT, a test with a cell density at start which was lower than the one recommended in guidelines, was dismissed.
- It is important to distinguish that the degradation of the isothiazolinones in the test is due to their reactivity with the test organisms, which also accounts for the toxicity.
- Initial effects after 24 h are typically seen, which are transient, and the NOEC therefore typically increase with time.

- In the case of DCOIT, a TWA approach could not be used as already after 24 h the substance was below its quantification limit (LoQ). The approach was therefore to base the NOEC on the effects after 24 h, and relate these to the initial measured concentration. At the time, and for PT21 (antifouling agents), this was considered as a conservative approach
- One MS reported that for *isothiazolinones* assessed by them the initial approach that has been taken is to use the NOEC after 48 h, and calculate a TWA concentration using concentration measured at the start of the test together with the LoQ / 2 at 48 h.
- The reactive *isothiazolinones* present a special case and might therefore warrant a deviation from guidelines (e.g. TWA). This needs to be clearly set down in the MoTA
- It can be questioned whether a NOEC after only 24 h can be considered as a measure of chronic toxicity, considering that no or only a few algal reproduction cycles have occurred in this time. It was noted that it is important that the minimum required growth rate in the controls are fulfilled.
- It is crucial that the time for which the NOEC is taken really represents the most sensitive point. In the case of MIT, NL asked for a second look at the results of the tests.

Results and data from algal tests and questions for e-consulation

It concerns the following four growth inhibition tests with algae in the MIT draft CAR.

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Reference**
			design	**duration**	
OECD 201, EEC C.3 US EPA FIFRA 122-2	*Pseudokirchneriella subcapitata*	120 h EC_{50}/NOEC	static	120 h	A7.4.1.3.a/01 (Rohm and Haas S.A.S.)
US EPA FIFRA 123-2 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96 h EC_{50}/NOEC	static	96 h	A7.4.1.3-01 (Thor GmbH)
US EPA FIFRA 123-2	*Skeletonema costatum*	120 h EC_{50}	static	120 h	A7.4.1.3.b/01 (Rohm and Haas S.A.S.)
ISO 10253 OPPTS 850.5400	*Skeletonema costatum*	96 h EC_{50}	static	96 h	A7.4.1.3-02 (Thor GmbH)

Study with *Pseudokirchneriella subcapitata* A7.4.1.3.a/01 (Rohm and Haas S.A.S.)

Detailed results for the study are given in Appendix A. Endpoints were given in the study report, but also derived following independent analysis of the raw data. The results are summarized below.

	Endpoints given in study report (mg/L)				*Endpoints independent analysis (mg/L)*			
	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$
24h	-	-	<0.0503	0.0894	0.1	0.104	0.06211	0.1025
48h	-	-	0.0934	0.138	0.05	0.0503	0.09199	0.1371
72h	0.05	0.0503	0.107	0.158	0.1	0.104	0.1016	0.156
96h	-	-	0.153	0.203	-	-	-	-
120h	0.05	0.0503	0.175	0.220	-	-	-	-

Observations:

1. The initial cell density of 10,000 cells/mL is in line with OECD 201 recommendations for the test species.

2. Initial measured concentrations are >80% of nominal. Concentration of test substance declined in all test systems, but most pronounced in the test systems with the lower MIT concentrations. At the lowest test concentration proposed as NOEC in the study report the MIT concentration was below LOD after 96 hours.

3. Cell density in the control cultures increased by a factor 46-49 over 72 hours and thus fulfilled the criterion of increase by a factor of at least 16. Exponential growth of algae in the control was not maintained after 72 hours. Results for 96 and 120 hours should not be used.

4. Variability of growth rate in the control is rather high for the first 24 hours. 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 201 (2006) and was not applicable at the time the study was performed (1997).

5. E_rC_{10} and E_rC_{50} values increase with time. Such a trend is not clear for the NOE_rC values. The 24h $E_rC_{10,ini}$ value lies below the statistical 24h NOE_rC_{ini}. The NOE_rC derivation for the first 24 hours has a low sensitivity due to a rather high variability of control performance and the fact that only three instead of six control replicates were used. There is on the other hand a clear dose response for the first 24 hours supporting the 24h $E_rC_{10,ini}$ value as the relevant endpoint.

6. The results at the 0.200 mg a.i./L (nom) test level show inhibitory effects on algal growth up to 72 hours following nearly complete inhibition of growth during the first 24 hours. This suggests a long-term effect but is most likely an artefact of the small size of the test system. The nearly complete inhibition of growth during the first 24 hours 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.

Study with *Pseudokirchneriella subcapitata* A7.4.1.3-01 (Thor GmbH)

Detailed results for the study are given in Appendix B. Endpoints in the study report were derived based on mean measured concentrations. Endpoints based on initial measured concentrations were derived following independent analysis of the raw data. The results are summarized below.

Endpoints given in study report (mg/L) *Endpoints independent analysis (mg/L)*

	NOE_rC_{nom}	NOE_rC_{mm}	$E_rC_{10,mm}$	$E_rC_{50,mm}$	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$
24h	-	-	-	-	0.049	0.01	0.02331	0.1136
48h	-	-	-	-	0.049	0.01	0.04293	0.1184
72h	-	-	-	-	0.098	0.03	0.07424	0.1599
96h	-	0.12	-	0.23	-	-	-	-
120h	-	-	-	-	-	-	-	-

RMS observations:

1. The initial cell density of 10,000 cells/mL is in line with OECD 201 recommendations for the test species.

2. Initial measured concentrations are in the range of 16 to 172 % of nominal. Initial measured concentrations below <80% of nominal for the three lowest test concentrations suggest substantial degradation of MIT before the test systems were sampled. Concentration of test substance declined in all test systems, but most pronounced in the test systems with the lower test concentrations. Except for the two highest test concentrations, MIT concentration was in all test systems below LOD after 96 hours.

3. Cell density in the control cultures increased by a factor 84-109 over 72 hours and thus fulfilled the criterion of increase by a factor of at least 16. Exponential growth of algae in the control was not maintained after 72 hours. Results for 96 hours should not be used.

4. Variability of growth rate in the control is small for the first 24 hours. The test fulfils 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%.

5. E_rC_{10}, E_rC_{50} and NOE_rC values increase with time. The 24h $E_rC_{10,ini}$ value is similar to the statistical 24h NOE_rC_{ini}. The data for 0-24h result in a clearer dose-response compared to the data over 0-48h and 0-72h.

Study with *Skeletonema costatum* A7.4.1.3.b/01 (Rohm and Haas S.A.S.)

Detailed results for the study are given in Appendix C. Endpoints in the study report were derived based on initial measured concentrations. Endpoints based on initial measured concentrations were also derived following independent analysis of the raw data. The results are summarized below.

	Endpoints given in study report (mg/L)				*Endpoints independent analysis (mg/L)*			
	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$
24h	0.1	0.0725	-	0.0448	0.1	0.0725	0.04421	0.06948
48h	0.05	0.0358	-	≥0.0725	0.05	0.0358	0.0406	0.07963
72h	0.1	0.0725	-	≥0.0725	0.05	0.0358	~ 0.07083	~ 0.07481
96h	0.1	0.0725	-	≥0.0725	-	-	-	-

RMS observations:

1. The initial cell density of 10,000 cells/mL is comparable with standard studies done with the freshwater alga *Pseudokierchneriella subcapitata*.

2. Initial measured concentrations are 68-89% of nominal. Concentration of MIT declined in all test systems. At the lower test concentrations including the NOEC level the MIT concentration was below LOD after 96 hours.

3. Cell density in the control cultures increased by a factor 27-39 over 72 hours and thus fulfilled the criterion of increase by a factor of at least 16.

4. Variability of growth rate in the control is high for the first 24 hours. However, the test fulfils 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%.

5. E_rC_{10} and E_rC_{50} values increase with time. Such a trend is not clear for the N_rOEC values. The 24h $EC_{10,ini}$ value is far below the statistical 24h $NOEC_{ini}$. The NOEC derivation for the first 24 hours has a low sensitivity due to a high variability of control performance and the fact that only three instead of six control replicates were used. There is on the other hand a clear dose response for the first 24 hours supporting the 24h $E_rC_{10,ini}$ value as relevant endpoint.

6. The results at the 0.100 mg a.i./L nominal test level suggest inhibitory effects on algal growth up to 48 hours following strong inhibition of growth during the first 24 hours. 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 hours 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.

Study with *Skeletonema costatum* A7.4.1.3-02 (Thor GmbH)

Detailed results for the study are given in Appendix D.

The study report states: *This organism forms chains containing several cells. As the electronic particle counter, used for the cell density determination, count particles and not individual cells, 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 it was decided to base the calculation of the endpoints on the algal biovolume in the cultures.*

Endpoints in the study report were derived based on mean measured concentration. Endpoints based on initial measured concentrations were derived following independent analysis of the raw data on algal biovolumes in each replicate. The results are summarized below.

	Endpoints given in study report (mg/L)				*Endpoints independent analysis (mg/L)*			
	NOE_rC_{nom}	NOE_rC_{mm}	$E_rC_{10,mm}$	$E_rC_{50,mm}$	NOE_rC_{nom}	NOE_rC_{ini}	$E_rC_{10,ini}$	$E_rC_{50,ini}$
24h	-		-	-	0.0112	0.0113	0.0727	0.1945
48h	-	-	-	-	0.0357	0.036	0.1046	0.1412
72h	-	0.0379	0.040	0.099	0.112	0.1057	~0.2446	~0.3270
96h	-	0.0379	0.055	0.0991	-	-	-	-

RMS observations:

1. An initial cell density of 1,000 cells/mL is not comparable with standard studies done with the freshwater alga *Pseudokierchneriella subcapitata*.

2. Initial measured concentrations are 94-104% of nominal. Based on the analytical results after 72 hours concentration of MIT declined in all test systems, but most pronounced in the test systems with the lower test concentrations (MIT concentration below LOD after 72 hours for the two lowest test concentrations). However, analytical results after 96 h are contra dictionary.

3. Algal biovolume values in the control cultures increased by a factor 750-938 over 72 hours and thus fulfilled the criterion of increase by a factor of at least 16. The exceptional high growth rate is probably linked with the low initial cell density.

4. Variability of growth rate in the control is low for the first 24 hours. The test fulfils 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%.

5. E_rC_{10}, E_rC_{50} N_rOEC values increase with time. The NOEC derivation for the first 24 hours 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 hours compared to 48 and 72 hours.

6. The results at the 0.357 mg a.i./L nominal test level suggest inhibitory effects on algal growth up to 48 hours following strong inhibition of growth during the first 24 hours. 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 hours 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.

Appendix A Detailed results for study with the freshwater alga *Pseudokirchneriella subcapitata* A7.4.1.3.a/01 (Rohm and Haas S.A.S.)

Initial measured concentrations of MIT represented 89-104% of nominal concentrations. A table with analytical results is included below. Measured concentrations expressed as percentage of nominal are reported between brackets. This illustrates that the concentration of MIT declined in all test systems, but most pronounced in the test systems with the two lowest test concentrations.

Measured 0 h (mg ai/L)	Measured 72 h (mg ai/L)	Measured 120 h (mg ai/L)
Not detected* (control)	Not detected (control)	Not detected (control)
0.0503 (104)	Not detected (<20)	Not detected (<20)
0.104 (104)	0.0193 (19)	Not detected (<10)
0.202 (101)	0.127 (63)	0.0367 (18)
0.407 (102)	0.233 (58)	0.235 (59)
0.708 (89)	0.685 (86)	0.583 (73)

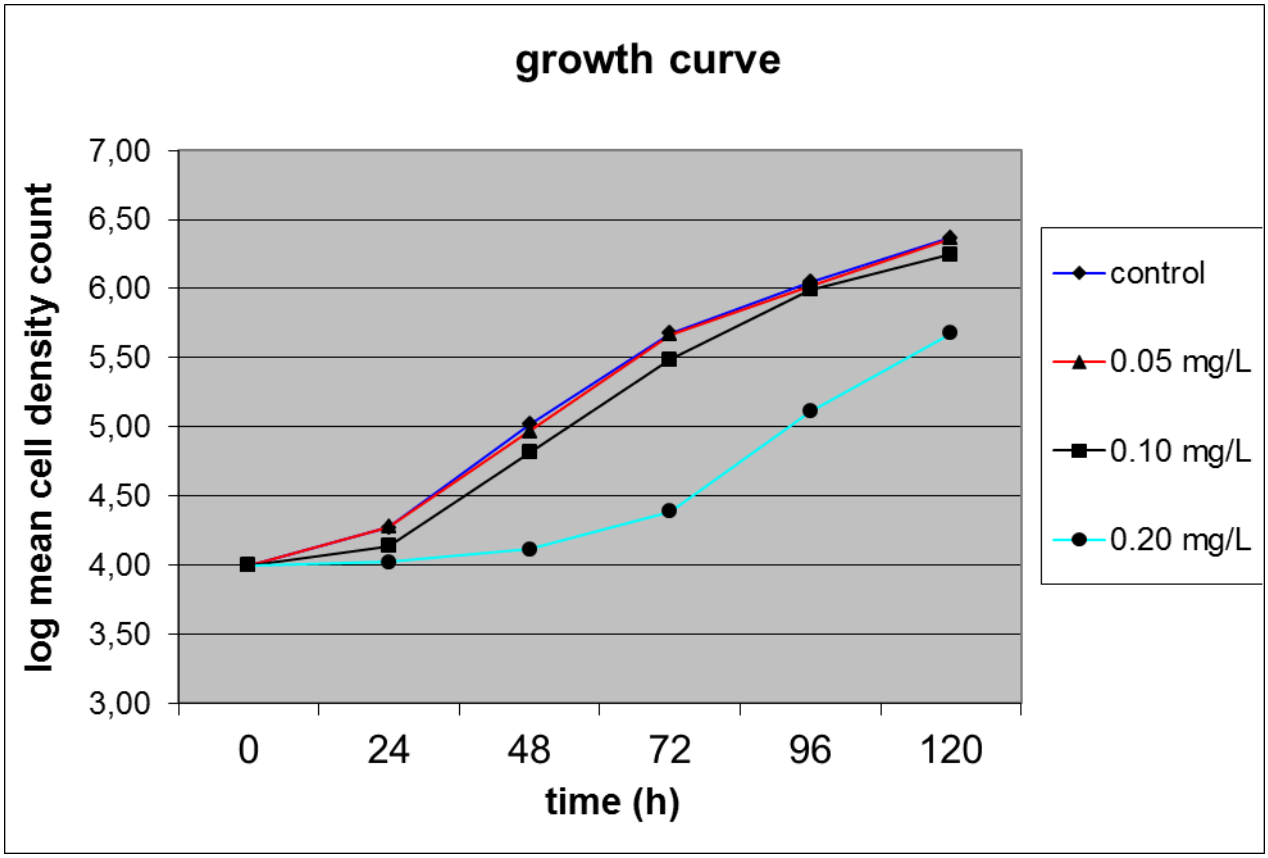
Cell concentration data for all replicates are given in the table below.

measured conc.	Repl.	Number of cells per millimeter					
		Hour of exposure					
0 h		0	24	48	72	96	120
<LOD	1	10000	16000	86000	490000	1284000	2526000
	2	10000	21000	102000	462000	1020000	2194000
	3	10000	19000	124000	476000	1054000	2290000
	Average	10000	18667	104000	476000	1119333	2336667
0,0503	1	10000	24000	82000	434000	950000	2446000
	2	10000	17000	92000	516000	1066000	2188000
	3	10000	16000	106000	440000	1086000	2280000
	Average	10000	19000	93333	463333	1034000	2304667
0,104	1	10000	11000	76000	310000	1062000	1652000
	2	10000	14000	56000	298000	862000	1734000
	3	10000	16000	64000	316000	1030000	1936000
	Average	10000	13667	65333	308000	984667	1774000
0,202	1	10000	11000	12000	14000	136000	468000
	2	10000	10000	17000	37000	122000	510000
	3	10000	<10000	10000	22000	130000	452000
	Average	10000	10500	13000	24333	129333	476667
0,407	1	10000	12000	10000	<10000	<10000	<10000
	2	10000	10000	<10000	<10000	<10000	<10000
	3	10000	<10000	<10000	<10000	<10000	<10000

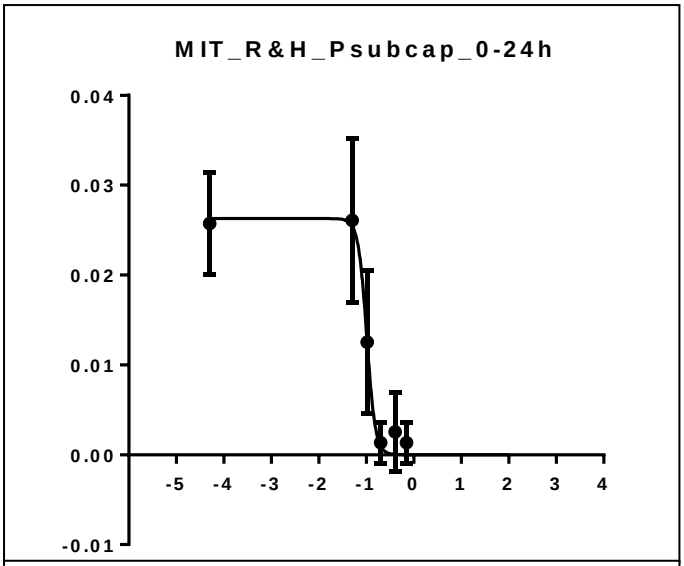
	Average	10000	<10500	<10000	<10000	<10000	<10000
0,708	1	10000	<10000	<10000	<10000	<10000	<10000
	2	10000	<10000	<10000	<10000	<10000	<10000
	3	10000	11000	<10000	<10000	<10000	<10000
	Average	10000	<10300	<10000	<10000	<10000	<10000

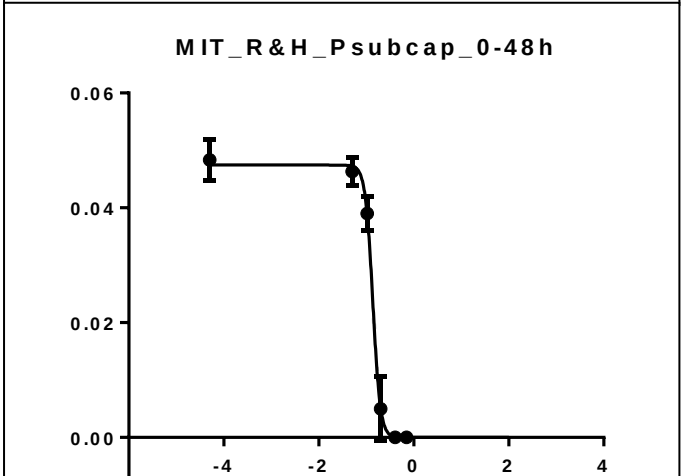
Based on cell concentration data given above the following specific growth rates were calculated. OECD 201 (2006) notes that a growth curve with cell density count plotted at a log scale generally gives a better presentation of variation in growth pattern during the test period. A log scale growth curve is given below.

Meas.conc.	Repl	Replicate and average specific growth rate						
		µ0-24	µ24-48	µ48-72	µ72-96	µ96-120	µ0-48	µ0-72
<LOD	1	0.020	0.070	0.073	0.040	0.028	0.045	0.054
	2	0.031	0.066	0.063	0.033	0.032	0.048	0.053
	3	0.027	0.078	0.056	0.033	0.032	0.052	0.054
	Average	0.026	0.072	0.063	0.036	0.031	0.049	0.054
0.0503	1	0.036	0.051	0.069	0.033	0.039	0.044	0.052
	2	0.022	0.070	0.072	0.030	0.030	0.046	0.055
	3	0.020	0.079	0.059	0.038	0.031	0.049	0.053
	Average	0.027	0.066	0.067	0.033	0.033	0.047	0.053
0.104	1	0.004	0.081	0.059	0.051	0.018	0.042	0.048
	2	0.014	0.058	0.070	0.044	0.029	0.036	0.047
	3	0.020	0.058	2.000	0.049	0.026	0.039	0.048
	Average	0.013	0.065	0.065	0.048	0.025	0.039	0.048
0.202	1	0.004	0.004	0.006	0.095	0.051	0.004	0.005
	2	0.000	0.022	0.032	0.050	0.060	0.011	0.018
	3	0.000	0.000	0.033	0.074	0.052	0.000	0.011
	Average	0.001	0.009	0.026	0.070	0.054	0.005	0.012
0.407	1	0.008	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Average	0.003	0.000	0.000	0.000	0.000	0.000	0.000
0.708	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.004	0.000	0.000	0.000	0.000	0.000	0.000
	Average	0.001	0.000	0.000	0.000	0.000	0.000	0.000



Dose response curves for 0-24h, 0-48h and 0-72 h data





Appendix B Detailed results for study with the freshwater alga *Pseudokirchneriella subcapitata* A7.4.1.3-01 (Thor GmbH)

Initial measured concentrations of MIT represented 16-122% of nominal concentrations. A table with analytical results is included below. Measured concentrations expressed as percentage of nominal are reported between brackets. This illustrates that the concentration of MIT declined in all test systems, but most pronounced in the test systems with the lower test concentrations.

TEST SUBSTANCE CONCENTRATION (MG/L) TEST ITEM / ACTIVE SUBSTANCE	**Measured 0 h (mg test item/L)**	**Measured 96 h (mg test item/L)**
Control	<LOD	<LOD
0.05 / 0.0245	0.004 (16)	<LOD (<98)
0.1 / 0.049	0.02 (41)	<LOD (<49)
0.2 / 0.098	0.06 (61)	<LOD (<24)
0.4 / 0.196	0.24 (122)	<LOD (<12)
0.8 / 0.392	0.445 (114)	<LOD (<6)
1.6 / 0.784	0.845 (108)	0.14 (29)
3.2 / 1.568	1.605 (102)	1.015 (65)

Cell concentration data for all replicates are given in the table below.

Nominal conc.	Repl.	Number of cells per millimeter Hour of exposure				
		0	24	48	72	96
control	1	10625	60948	296712	846124	1146249
	2	10380	55485	305777	868787	1071524
	3	10588	61671	309697	969849	1099699
	4	10919	67257	381359	941062	1116849
	5	10294	60287	344242	922074	984549
	6	10343	54921	378174	1094595	1148699
	Mean	10525	60095	335994	940415	1094595
0.0245	1	10919	58596	300142	941062	1143799
	2	10147	66620	343752	933712	1081324
	3	12218	57432	345467	853474	1143799
	Mean	11095	60883	329787	909416	1122974
0.049	1	12022	54541	324029	938612	1084999
	2	10968	60127	319619	838774	1141524
	3	11054	57445	351592	965562	1163399
	Mean	11348	57371	331747	914316	1129974
0.098	1	11225	43284	201529	702187	1076424

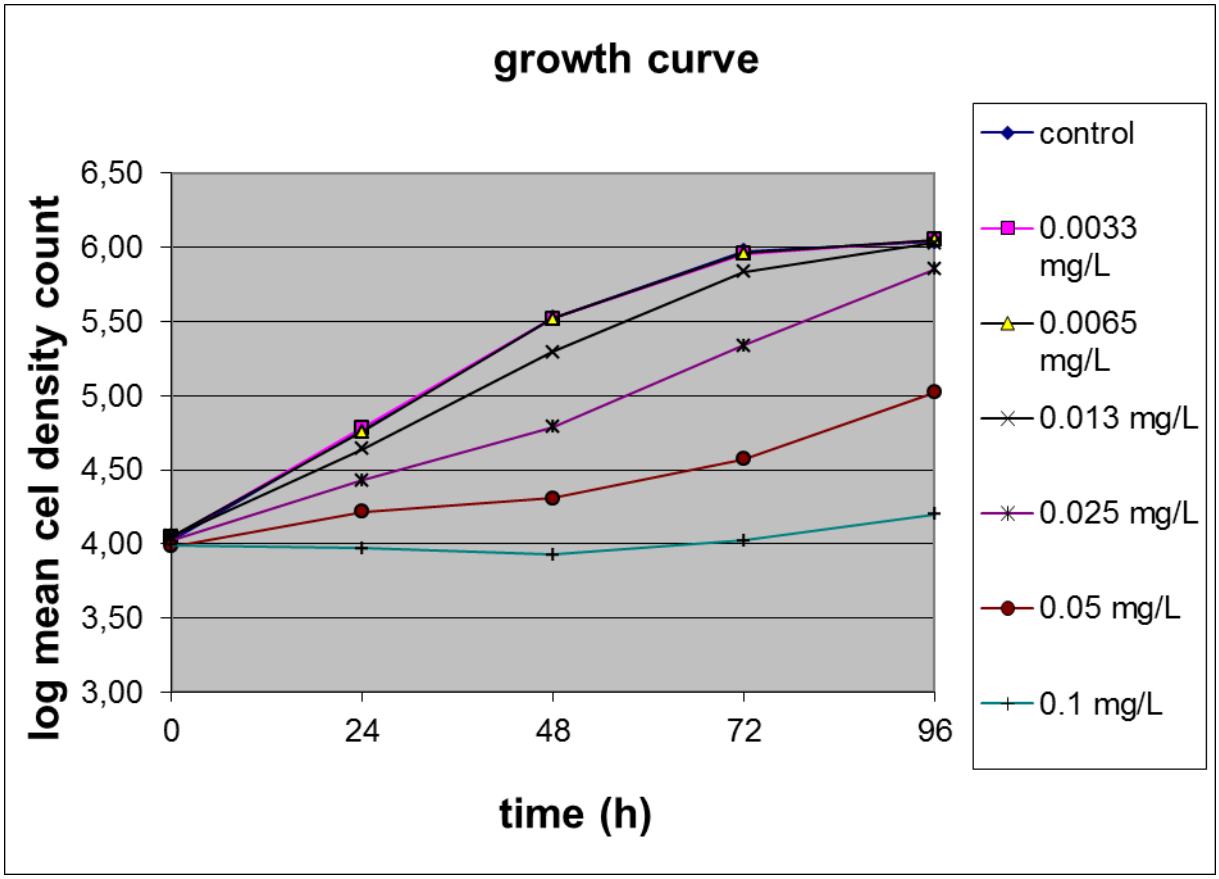
	2	11654	42622	189524	657474	1021299
	3	11140	46028	198834	699737	1115624
	Mean	11340	43978	196629	686466	1071116
0.196	1	11127	25154	60017	209247	704024
	2	10074	26844	63178	232522	735262
	3	10870	28547	61169	210227	694837
	Mean	10690	26848	61455	217332	711374
0.392	1	8653	15942	22765	44607	145645
	2	10380	17730	21503	40772	109801
	3	9792	15733	16860	26477	59086
	Mean	9608	16468	20376	37285	104844
0.49	1	9743	9755	10147	13626	18171
	2	10023	8567	7906	9915	16493
	3	9645	9717	7269	7942	12940
	Mean	9804	9346	8441	10494	15868
1.568	1	9694	10625	5125	3091	4194
	2	9008	10331	3606	2283	1376
	3	8751	11495	7036	5652	6080
	Mean	9151	10625	5256	3675	3883

Based on cell concentration data given above the following specific growth rates were calculated.

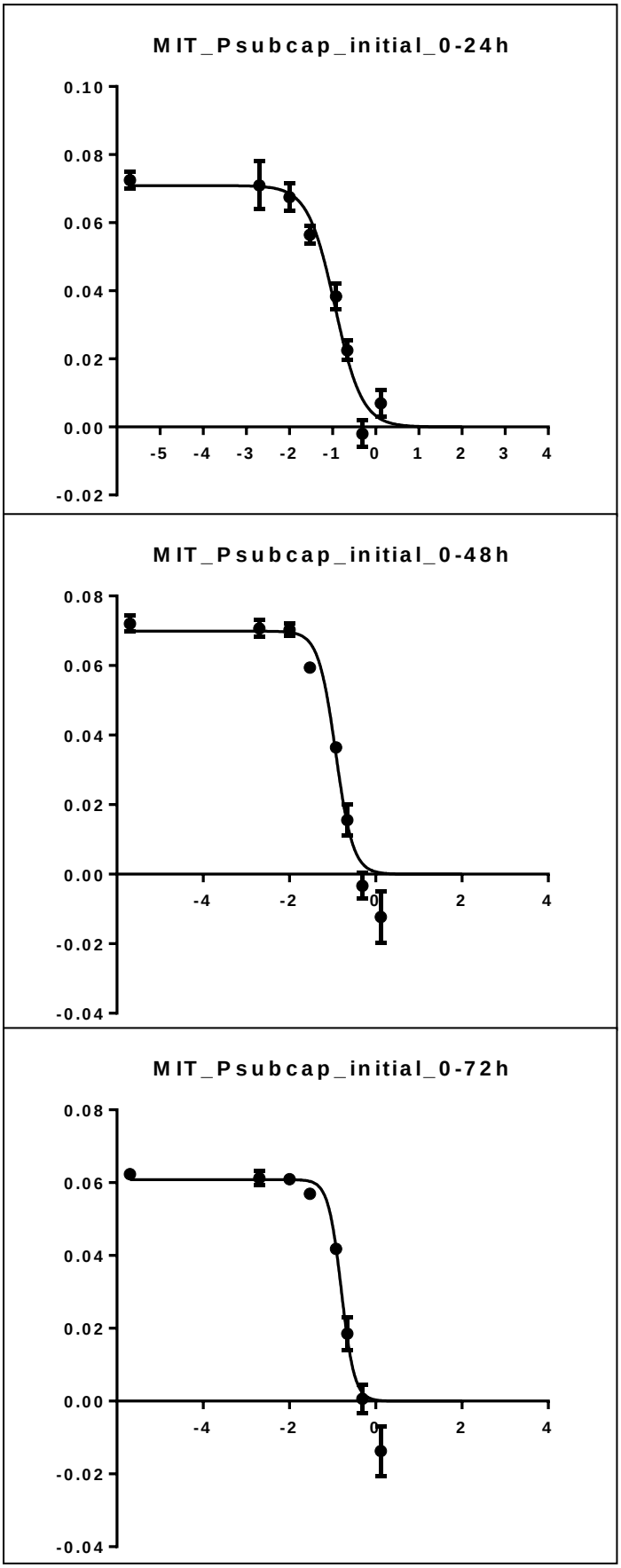
Nominal conc.	Rep	Average specific growth rate						
		µ0-24	µ24-48	µ48-72	µ72-96	µ0-48	µ0-72	µ0-96
control	1	0.073	0.066	0.044	0.013	0.069	0.061	0.049
	2	0.070	0.071	0.044	0.009	0.070	0.061	0.048
	3	0.073	0.067	0.048	0.005	0.070	0.063	0.048
	4	0.076	0.072	0.038	0.007	0.074	0.062	0.048
	5	0.074	0.073	0.041	0.003	0.073	0.062	0.048
	6	0.070	0.080	0.044	0.002	0.075	0.065	0.049
	Mean	0.073	0.072	0.043	0.006	0.072	0.062	0.048
0.0245	1	0.070	0.068	0.048	0.008	0.069	0.062	0.048
	2	0.078	0.068	0.042	0.006	0.073	0.063	0.049
	3	0.064	0.075	0.038	0.012	0.070	0.059	0.047
	Mean	0.071	0.070	0.042	0.009	0.071	0.061	0.048
0.049	1	0.063	0.074	0.044	0.006	0.069	0.061	0.047
	2	0.071	0.070	0.040	0.013	0.070	0.060	0.048
	3	0.069	0.075	0.042	0.008	0.072	0.062	0.049
	Mean	0.068	0.073	0.042	0.009	0.070	0.061	0.048
0.098	1	0.056	0.064	0.052	0.018	0.060	0.057	0.048
	2	0.054	0.062	0.052	0.018	0.058	0.056	0.047
	3	0.059	0.061	0.052	0.019	0.060	0.058	0.048
	Mean	0.056	0.062	0.052	0.019	0.059	0.057	0.047
0.196	1	0.034	0.036	0.052	0.051	0.035	0.041	0.043
	2	0.041	0.036	0.054	0.048	0.038	0.044	0.045
	3	0.040	0.032	0.051	0.050	0.036	0.041	0.043

	Mean	0.038	0.035	0.053	0.049	0.036	0.042	0.044
0.392	1	0.025	0.015	0.028	0.049	0.020	0.023	0.029
	2	0.022	0.008	0.027	0.041	0.015	0.019	0.025
	3	0.020	0.003	0.019	0.033	0.011	0.014	0.019
	Mean	0.023	0.009	0.024	0.041	0.016	0.019	0.025
0.49	1	0.000	0.002	0.012	0.012	0.001	0.005	0.006
	2	0.000	0.000	0.009	0.021	0.000	0.000	0.005
	3	0.000	0.000	0.004	0.020	0.000	0.000	0.003
	Mean	0.000	0.001	0.008	0.018	0.000	0.001	0.005

OECD 201 (2006) notes that a growth curve with cell density count plotted at a log scale generally gives a better presentation of variation in growth pattern during the test period. A log scale growth curve is given below.


growth curve
log mean cel density count
6,50
6,00
5,50
5,00
4,50
4,00
3,50
3,00
0
24
48
72
96
time (h)
control
0.0033 mg/L
0.0065 mg/L
0.013 mg/L
0.025 mg/L
0.05 mg/L
0.1 mg/L

Dose response curves for 0-24h, 0-48h and 0-72 h data



Appendix C Detailed results for study with the marine alga *Skeletonema costatum* A7.4.1.3.a/01 (Rohm and Haas S.A.S.)

Initial measured concentrations of MIT represented 68-89% of nominal concentrations. A table with analytical results is included below. Measured concentrations expressed as percentage of nominal are reported between brackets. This illustrates that the concentration of MIT declined in all test systems.

Measured 0 h (mg ai/L)	Measured 96 h (mg ai/L)
Not detected* (control)	Not detected (control)
0.00294 (89)	Not detected (<64)
0.00476 (73)	Not detected (<33)
0.00948 (73)	Not detected (<16)
0.0170 (68)	Not detected (<8)
0.0358 (72)	0.00484 (10)
0.0725 (73)	0.00669 (7)

Cell concentration data for all replicates are given in the table below.

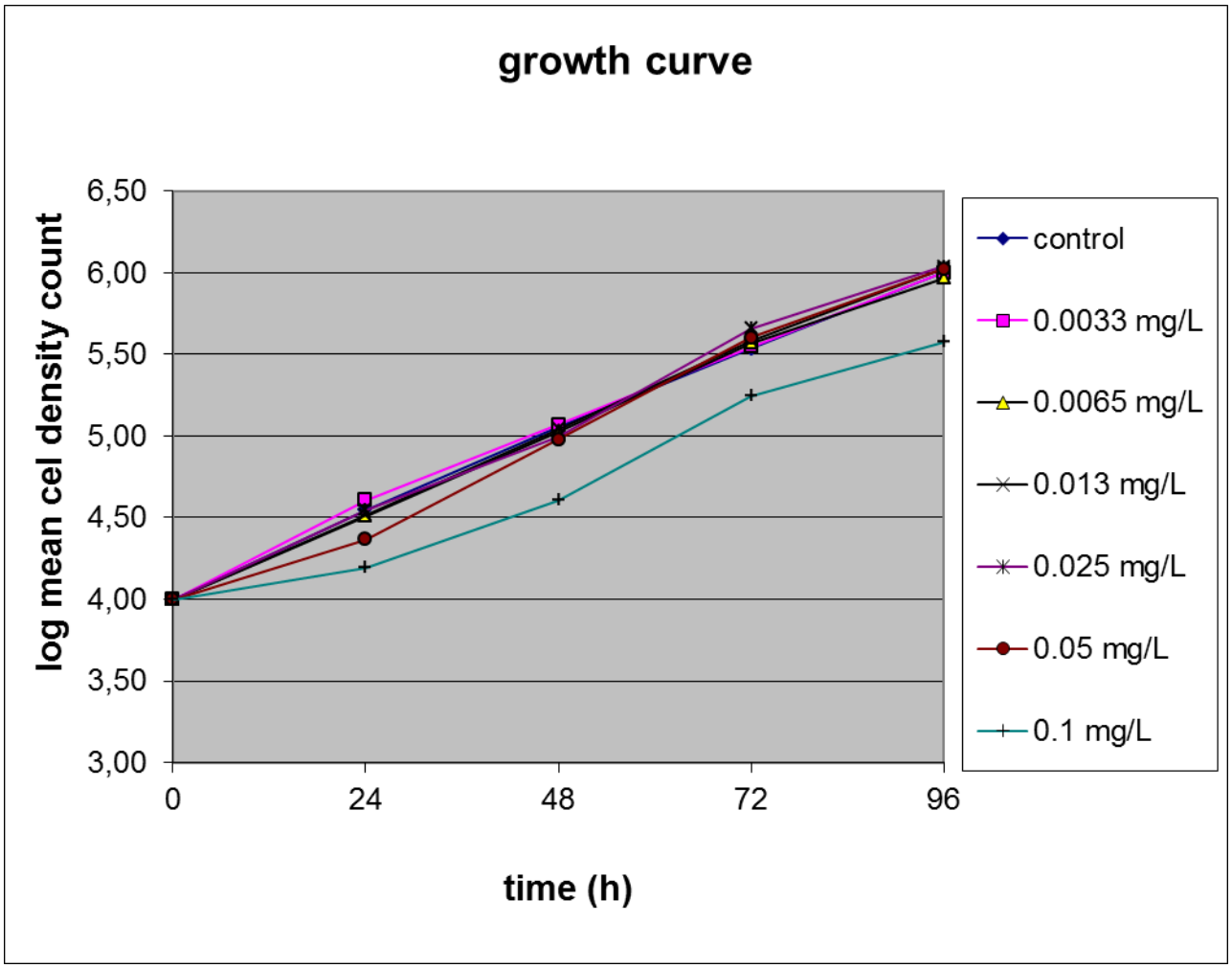
Nominal conc.	measured conc.	Repl.	Number of cells per millimeter Hour of exposure				
	0 h		0	24	48	72	96
control	<LOQ	1	10000	21000	110000	270000	970000
		2	10000	52000	110000	380000	970000
		3	10000	33000	120000	380000	1080000
		Mean	10000	35333	113333	343333	1006667
0.0033	0.00294	1	10000	47000	130000	310000	1060000
		2	10000	17000	130000	460000	980000
		3	10000	57000	91000	290000	970000
		Mean	10000	40333	117000	353333	1003333
0.0065	0.00476	1	10000	14000	100000	300000	790000
		2	10000	40000	140000	450000	1030000
		3	10000	43000	81000	360000	970000
		Mean	10000	32333	107000	370000	930000
0.013	0.00948	1	10000	38000	110000	330000	950000
		2	10000	42000	110000	440000	1020000
		3	10000	19000	110000	380000	1190000
		Mean	10000	33000	110000	383333	1053333
0.025	0.017	1	10000	40000	110000	400000	1140000
		2	10000	47000	94000	470000	1300000
		3	10000	19000	94000	500000	840000
		Mean	10000	35333	99333	456667	1093333
0.05	0.0358	1	10000	29000	97000	520000	990000

		2	10000	7800	78000	350000	1050000
		3	10000	33000	110000	330000	1130000
		Mean	10000	23267	95000	400000	1056667
0.1	0.0725	1	10000	17000	31000	140000	370000
		2	10000	22000	52000	210000	430000
		3	10000	7800	38000	180000	330000
		Mean	10000	15600	40333	176667	376667

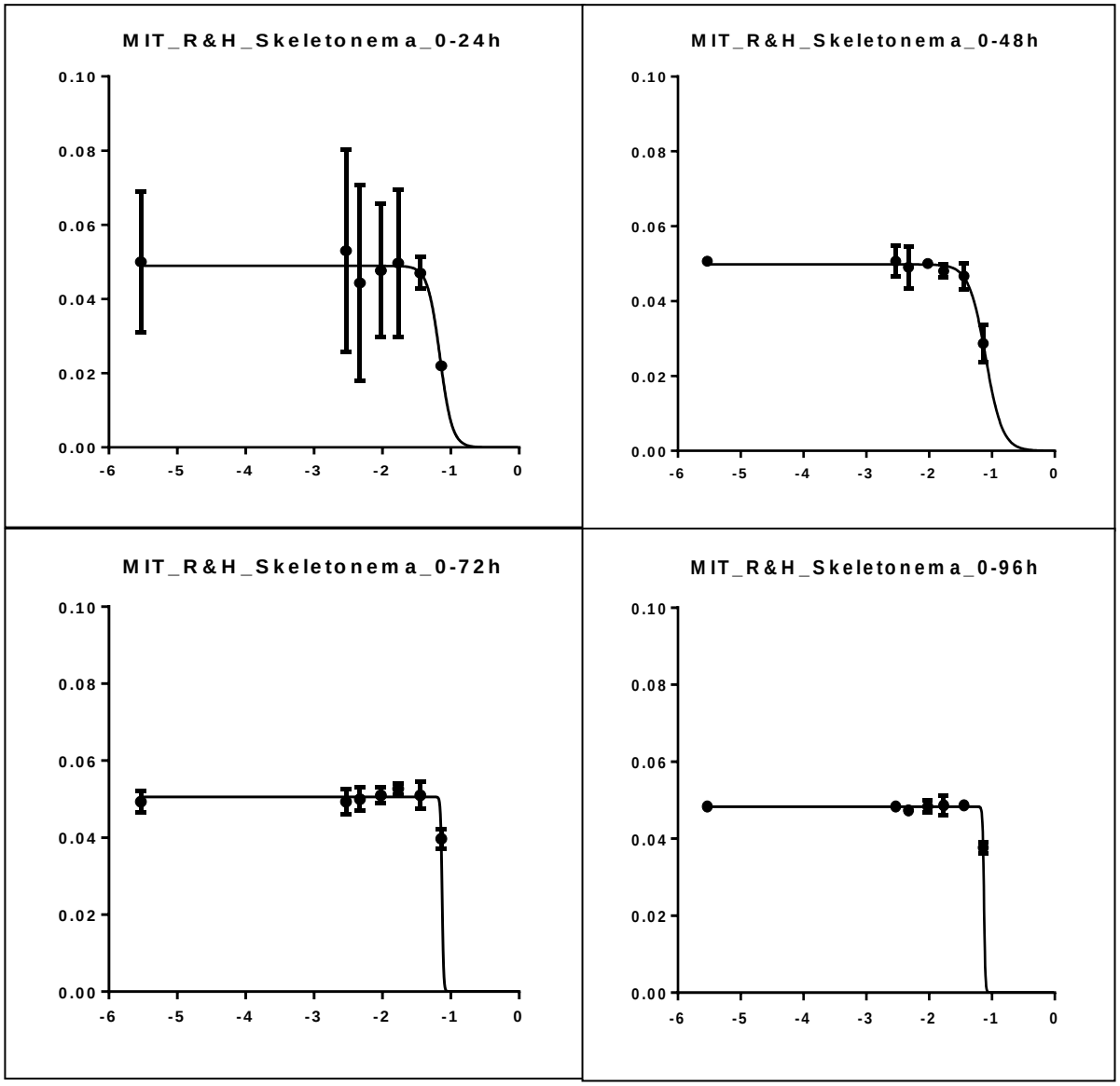
Based on cell concentration data given above the following specific growth rates were calculated.

Nominal conc	Measured 0 h	Repl.	Average specific growth rate						
			µ0-24	µ24-48	µ48-72	µ72-96	µ0-48	µ0-72	µ0-96
control	<LOQ	1	0.031	0.069	0.037	0.053	0.050	0.046	0.048
		2	0.069	0.031	0.052	0.039	0.050	0.051	0.048
		3	0.050	0.054	0.048	0.044	0.052	0.051	0.049
		Mean	0.050	0.051	0.046	0.045	0.051	0.049	0.048
0.0033	0.00294	1	0.064	0.042	0.036	0.051	0.053	0.048	0.049
		2	0.022	0.085	0.053	0.032	0.053	0.053	0.048
		3	0.073	0.019	0.048	0.050	0.046	0.047	0.048
		Mean	0.053	0.049	0.046	0.044	0.051	0.050	0.048
0.0065	0.00476	1	0.014	0.082	0.046	0.040	0.048	0.047	0.046
		2	0.058	0.052	0.049	0.035	0.055	0.053	0.048
		3	0.061	0.026	2.000	0.041	0.044	0.050	0.048
		Mean	0.044	0.054	0.698	0.039	0.049	0.050	0.047
0.013	0.00948	1	0.056	0.044	0.046	0.044	0.050	0.049	0.047
		2	0.060	0.040	0.058	0.035	0.050	0.053	0.048
		3	0.027	0.073	0.052	0.048	0.050	0.051	0.050
		Mean	0.047	0.053	0.052	0.042	0.050	0.051	0.049
0.025	0.017	1	0.058	0.042	0.054	0.044	0.050	0.051	0.049
		2	0.064	0.029	0.067	0.042	0.047	0.053	0.051
		3	0.027	0.067	0.070	0.022	0.047	0.054	0.046
		Mean	0.050	0.046	0.063	0.036	0.048	0.053	0.049
0.05	0.0358	1	0.044	0.050	0.070	0.027	0.047	0.055	0.048
		2	0.000	0.096	0.063	0.046	0.043	0.049	0.048
		3	0.050	0.050	0.046	0.051	0.050	0.049	0.049
		Mean	0.031	0.065	0.059	0.041	0.047	0.051	0.049
0.1	0.0725	1	0.022	0.025	0.063	0.040	0.024	0.037	0.038
		2	0.033	0.036	0.058	0.030	0.034	0.042	0.039
		3	0.000	0.066	0.065	0.025	0.028	0.040	0.036
			0.018	0.042	0.062	0.032	0.029	0.040	0.038

OECD 201 (2006) notes that a growth curve with cell density count plotted at a log scale generally gives a better presentation of variation in growth pattern during the test period. A log scale growth curve is given below.



Dose response curves for 0-24h, 0-48h, 0-72 h and 0-96h data



Appendix D Detailed results for study with the marine alga *Skeletonema costatum* A7.4.1.3-02 (Thor GmbH)

Initial measured concentrations of MIT represented 94-104% of nominal concentrations. A table with analytical results is included below. Concentration of MIT declined in all test systems.

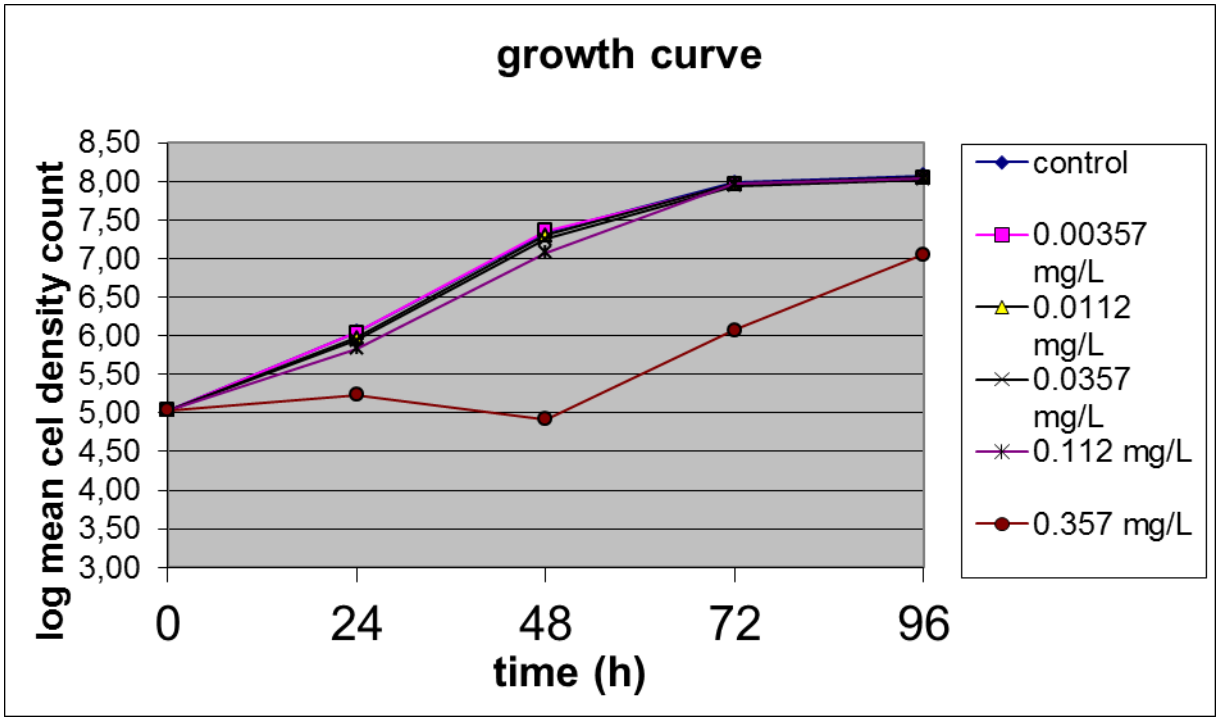
Nominal conc. MIT ($\mu g.l^{-1}$)	**Measured concentration MIT**					
	0 h		**72 h**		**96 h**	
	$\mu g.l^{-1}$	**% of nominal**	**$\mu g.l^{-1}$**	**% of start conc.**	**$\mu g.l^{-1}$**	**% of start conc.**
0	n.d.	-	n.d.	-	n.d.	-
3.57	3.7	104	n.d.	-	1.3	35.1
11.2	11.3	101	n.d.	-	3.6	31.9
35.7	36.0	101	1.6	4.4	7.2	20.0
112	105.7	94.4	10.7	10.1	7.2	6.8
357	356.7	99.9	56.0	15.7	46.2	13.0

Algal biovolume data corrected for background values for all replicates are given in the table below.

Nominal conc.	measured conc.	Repl.	Number of cells per millimeter				
			Hour of exposure				
	0 h		0	24	48	72	96
control	<LOQ	1	107900	1021813	23471300	101217800	134723300
		2	107900	1129854	22571100	87964200	103708100
		3	107900	1164613	23741300	112527800	135323300
		4	107900	1179854	20531100	92754200	113808100
		5	107900	1092613	19941300	80957800	108123300
		6	107900	1082854	21231100	97204200	111508100
		Average	107900	1111934	21914533	95437667	117865700
0.00357	0.0037	1	107900	1111748	21740600	88236900	102353500
		2	107900	1017748	22280600	88506900	111253500
		3	107900	1151748	24870600	92776900	114553500
		Average	107900	1093748	22963933	89840233	109386833
0.0112	0.0113	1	107900	850600	17066200	82852600	107367000
		2	107900	903200	20726200	93602600	115967000
		3	107900	1056900	22176200	99682600	115867000

		Average	107900	936900	19989533	92045933	113067000
0.0357	0.036	1	107900	857702	19427200	87218100	104470000
		2	107900	711402	14897200	90778100	108770000
		3	107900	1018902	18787200	85573200	101670000
		Average	107900	862669	17703867	87856467	104970000
0.112	0.1057	1	107900	649152	13951300	102543200	119577600
		2	107900	707752	11901300	85573200	108577600
		3	107900	673752	10041300	88373200	100577600
		Average	107900	676885	11964633	92163200	109577600
0.357	0.3567	1	107900	195750	78700	1847700	15445000
		2	107900	181850	100500	895700	9745000
		3	107900	134550	68400	782700	8444000
		Average	107900	170717	82533	1175367	11211333

OECD 201 (2006) notes that a growth curve with cell density count plotted at a log scale generally gives a better presentation of variation in growth pattern during the test period. A log scale growth curve is given below.



Dose response curves for 0-24h, 0-48h and 0-72 h data