Committee for Risk Assessment

RAC

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

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

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

CLH-O-0000007341-81-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It is based on the official CLH report submitted to consultation and additional information (if applicable).

Adopted

14 September 2023

REGULATION (EC) NO 1272/2008 (CLP REGULATION),

ANNEX VI, PART 2

Proposal for Harmonised Classification and Labelling for a biocidal active substance

CLH REPORT

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

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

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

Index Number: **Not applicable**

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Version number: 3.0 **Date: September2022**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

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STATEMENT OF SUBJECT MATTER AND PURPOSE OF THE CAR

Not applicable for the CLH report.

ASSESSMENT REPORT

SUMMARY

1. PRESENTATION OF THE ACTIVE SUBSTANCE

1.1 IDENTITY OF THE ACTIVE SUBSTANCE

Table 1.1 Main constitiuents

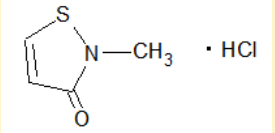
Main constituent(s)	
ISO name	2-methyl-2H-isothiazol-3-one hydrochloride N-MIT·HCl 2-Methyl-4-isothiazolin-3-one hydrochloride
IUPAC or EC name	IUPAC: 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride EC: 2-methyl-2H-isothiazol-3-one hydrochloride
EC number	247-499-3
CAS number	26172-54-3
Index number in Annex VI of CLP	Not applicable
Minimum purity / content	>99 – ≤100%
Structural formula	

Table 1.2 Relevant impurities and additives

Relevant impurities and additives		
IUPAC name or chemical name or EC name	Maximum concentration in % (w/w)	Index number in Annex VI of CLP
Please refer to the APPENDIX VI: CONFIDENTIAL INFORMATION.		

1.2 INTENDED USES AND EFFECTIVENESS

Table 1.3 Use of the active substance

Product type	PT 6: Preservatives for products during storage PT 6.7: Mineral slurries and other matrices
Intended use pattern(s)	Active substance is intended to be used as a preservative in aqueous solutions in life science, specifically in reagents for scientific research and

	development in life science sector and in the production of biomolecules. The preserved products may be biopharmaceuticals, medical devices, nutraceuticals, or tools for forensic sciences or molecular biology including both large industrial installations and small and medium laboratories. • used as preservative in different water-based solutions like antibody-, protein solutions used for protein isolation and separation and isolation steps in immunoassays as well as nucleic acid preparations • used as preservative in buffer solutions for medical and scientific research market in a controlled laboratory environment • used as preservative in different filtration membranes and filters • used as preservative in chromatography resins, which are used to purify proteins and nucleic acids in a variety of life sciences applications, such as R&D and manufacturing of human therapeutics, vaccines, gene therapy medicines and somatic-cell therapy medicines
Users	Professional/Industrial

Table 1.4 Effectiveness of the active substance

Function	N-MIT·HCl is a preservative
Organisms to be controlled	Bacteria, yeasts and fungi
Limitation of efficacy including resistance	Factors that may influence efficacy but are not considered in efficacy tests, such as pH, interfering substances, are not known to be limiting the efficacy of N-MIT. Microbial resistance to N-MIT should not present a significant problem in theory. Namely, the mechanism of action of N-MIT affects a variety of metabolic processes within the cell. It is very difficult for microorganisms to develop the resistance against multiple targets simultaneously. Such an act would demand a significant amount of energy from microorganisms. Nevertheless, a report of microbial resistance attributed to N-MIT active ingredient from a laboratory adapted strain exists in the literature. It is important to emphasize that the use of preservatives induces a continuous contact between active substances and microorganisms, leading to a pressure of selection that could maintain the adapted resistant state of microorganisms. It is also reported in the literature that microorganisms deemed resistant to isothiazolones have also shown a varying cross-resistance to other biocides and to antibiotics.
Mode of action	Pure/non aqueous N-MIT·HCl is only present in solid state immediately after manufacturing. Once N-MIT·HCl is in an aqueous solution it dissociates into N-MIT and HCl. Chloride and hydrogen ions originating

	from N-MIT·HCl do not represent a biologically significant amount and do not contribute to the overall efficacy at the used N-MIT·HCl concentrations. N-MIT in aqueous solution originating from N-MIT·HCl is chemically-indistinguishable from N-MIT in aqueous solution originating from N-MIT. In an aqueous environment the nitrogen atom in N-MIT can exist as a protonated ion (conjugated acid) or an unprotonated free base with hydrogen from surrounding water molecules being used as the source of protons. The extent of protonation depends on the pH of the local environment. The pH in solutions, where N-MIT is active, is from 6-8. The dominant form for N-MIT will be the unprotonated form at pH 6-8. Therefore, it is chemically equivalent to N-MIT. N-MIT utilizes a two-step mechanism involving rapid inhibition (within minutes) of growth and metabolism, followed by an irreversible cell damage resulting in loss of viability (within hours). Cells are inhibited by disruption of the metabolic pathways involving dehydrogenase enzymes and cell death results from the destruction of protein thiols and the production of free radicals.

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO THE CLP CRITERIA

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING FOR THE ACTIVE SUBSTANCE

Table 2.1 Proposed harmonised classification and labelling of the substance

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

Table 2.2 Reason for not proposing harmonised classification and labelling and the status under CLH public consultation

Hazard class	Reason for not proposing classification and labelling	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances and mixtures	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances and mixtures	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not applicable	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Data lacking (data waived based on corrosivity)	Yes
Acute toxicity via inhalation route	Data lacking (data waived based on corrosivity)	Yes
Skin corrosion/irritation	Harmonised classification proposed	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Data conclusive but not sufficient for classification	Yes
Skin sensitisation	Harmonised classification proposed	Yes

Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not applicable	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

2.1.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride is a new biocidal active substance, not previously discussed and/or agreed by the TC C&L (Dir. 67/548/EEC) and/or RAC (CLP Regulation).

2.2 PROPOSED CLASSIFICATION AND LABELLING AND PACKAGING FOR THE REPRESENTATIVE PRODUCT

Not applicable for the CLH report.

2.3 DATA SOURCES

The reference list is provided in Part D, Appendix V.

The main data source are data on 2-methyl-2H-isothiazol-3-one (MIT; EINECS 220-239-6; CAS No 2682-20-4) to which the Applicant has a Letter of Access. A justification for the read across from already submitted and approved N-MIT (free base) to the new active substance N-MIT·HCl has been submitted by using the ECHA Read-Across Assessment Framework (RAAF, 2017a). Document with a justification for the read across is available in Annex II (Appendix VIII: Read across between N-MIT·HCl and N-MIT). In addition the data from related REACH registration dossier have been reviewed.

Table 2.3 Current harmonised classification and labelling of the substance MIT:

Substance: MIT	**Index No**	**International Chemical Identification**	**EC No**	**CAS No**	**Classification** Hazard Class and Category Code(s)	Hazard statement Code(s)	**Labelling** Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)	**Specific Conc. Limits, M-factors, ATE values**
Current Annex VI entry	613-326-00-9	2-methylisothiazol-3(2*H*)-one	220-239-6	2682-20-4	Acute Tox. 2 Acute Tox. 3 Acute Tox. 3 Skin corr. 1B Skin sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H311 H301 H314 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H311 H330 H314 H317 H410	EUH071	H317: SCL 0.0015% M=10 M=1

3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

3.1 SUMMARY OF THE ASSESSMENT OF EFFECTS ON HUMAN HEALTH

Not applicable for the CLH report.

3.2 REFERENCE VALUES

Not applicable for the CLH report.

3.3 RISK CHARACTERISATION

Not applicable for the CLH report.

4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

Not applicable for the CLH report.

4.1 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Not applicable for the CLH report.

4.2 EFFECTS ASSESSMENT

Not applicable for the CLH report.

4.3 EXPOSURE ASSESSMENT

Not applicable for the CLH report.

4.4 RISK CHARACTERISATION

Not applicable for the CLH report.

5. ASSESSMENT OF EXCLUSION CRITERIA, SUBSTITUTION CRITERIA AND POP

Not applicable for the CLH report.

A. Assessment of intrinsic properties and effects of the active substance

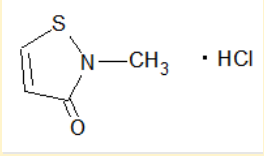
A.1. General substance information

A.1.1. Identity of the substance

Table A.1 Summary table on substance identity

Summary table on substance identity	
Common name (ISO name, synonyms)	2-methyl-2H-isothiazol-3-one hydrochloride N-MIT·HCl 2-Methyl-4-isothiazolin-3-one hydrochloride
Chemical name (EC name, CA name, IUPAC name)	IUPAC: 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride EC: 2-methyl-2H-isothiazol-3-one hydrochloride
EC number	247-499-3
CAS number	26172-54-3
other CAS numbers (e.g. deleted, related, preferred, alternate)	Not applicable
Molecular formula	$C_4H_5NOS \cdot HCl$
Molecular weight or molecular weight range	151.6 g/mol
Information on optical activity and typical ratio of (stereo) isomers	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%)*	

Table A.2 Structural formula

Structural formula


A.1.2. Composition of the substance (reference specifications)

Table A.3 Main constituents

Main constituents					
Constituent (chemical	Typical concentratio	Concentration range	Current CLH in Annex VI	Current self-classification	Remarks / Discussion

name)	n (%(w/w))	(%(w/w))	Table 3.1 (CLP)	and labelling (CLP)	
2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride	99.5%	>99 - ≤100%	No current harmonised classification	Acute Tox. 3, H301 Skin Corr. 1A, H314 Eye Dam. 1, H318 Skin Sens. 1A, H317 Acute Aquatic 1, H400 Acute Chronic 1, H410 EUH071	

Table A.4 Impurities

Impurities					
Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
No relevant impurities for classification and labelling.					

Table A.5 Additives

Additives						
Constituent (chemical name)	Function	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
Not applicable						

A.1.3. Physical and chemical properties of the active substance

Table A.6 Physical and chemical properties of the active substance

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Aggregate state at 20°C and 101.3 kPA	Solid	EPA OPPTS 830.630	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017a
Physical state (appearance) at 20°C and 101.3 kPA	Solid	EPA OPPTS 830.630	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017a
Colour at 20°C and 101.3 kPA	White	EPA OPPTS 830.6302 (Colour)	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017a
Odour at 20°C and 101.3 kPA	Characteristic	EPA OPPTS 830.6304 (Odor)	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017a
Acidity / alkalinity	pH = 1.30 (1% solution, 20 °C) Acidity was performed in deionised water and calculated to be 36% at 20 °C	CIPAC MT 75.3 CIPAC MT 191 (Acidity or Alkalinity of Formulations)	Combined glass electrode Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017a Fiesler, 2017a
Melting / freezing point	Melted in the range 165.0 °C to 170.0°C	OECD Guideline 102 EU Method A.1 EPA OPPTS 830.7200 (Melting Point / Melting Range)	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017b
Boiling point	No boiling point observed up to 400 °C; atmospheric pressure was 99.04 kPa	OECD Guideline 103 EU Method A.2 EPA OPPTS 830.7220 (Boiling Point / Boiling Range)	Capillary method using electronic controlled heating system Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017d
Relative density	1.551	OECD Guideline 109 EU Method A.3 EPA OPPTS 830.7300	Method: Air comparison pycnometer (for solids) Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017e
Infrared spectroscopy (FT-IR)	Conforms to structure	ATR	Test material: N-MIT·HCl Batch No.: 10013913,	Ruckstuhl, 2019

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			10012389, 27103200, 34050100 34050400	
NMR	Conforms to structure	^{1}H-NMR, ^{13}C-NMR	Test material: N-MIT·HCl Batch No.: 10013913 10012389, 34050100, 34050400	Ruckstuhl, 2019
UV/Vis spectroscopy	Max absorption (pure water solution): λ_{max}=207 nm: ε= 3003 [L·mol^{-1}·cm^{-1}] λ_{max}=274 nm: ε= 8067 [L·mol^{-1}·cm^{-1}]	OECD Guideline 101, Method: Molar extinction coefficient	Test material: N-MIT·HCl Batch No.: 10013913 10012389, 27103200, 34050100,34050400	Ruckstuhl, 2019
GC-MS	Conforms to structure	Mass spectrometry (MS)	Test material: N-MIT·HCl Batch No.: 10013913, 10012389, 27103200, 34050100, 34050400, Purity 99.9 %	Ruckstuhl, 2019
Granulometry	L50 = 105.0 µm σ_g = 3.7	CIPAC MT 187 OECD Guideline 110 ISO 13320	Method: laser scattering/diffraction Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Ahrens, 2018
Vapour pressure	7.77 × 10^{-1} Pa at 20 °C 1.00 Pa at 25 °C	OECD Guideline 104 EU Method A.4	Effusion method: isothermal thermogravimetry Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017f
Henry's law constant	2.8 x 10^{-4} Pa.m^3.mol^{-1}	Calculation based on GLP compliant study (Wagner, 2017f)	Calculation method	Moseley, 2018
Surface tension	71.3 mN/m at 20 °C	OECD Guideline 115 EU Method A.5	Ring method. An aqueous solution of 1 g/l Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wagner, 2017g
Water solubility at 20	420.3 g/l at 20 ± 0.5 °C,	OECD Guideline 105	The shake flask method was used,	Iffland, 2017

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
°C	pH 0	EU Method A.6	because the solubility determined in the preliminary test was above 10 mg/L. Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	
Water solubility (effect of temperature and pH)	Not applicable	Waiver: study scientifically not necessary / other information available The test item reacts highly acidic in water (pKa of test item is 2.513). Therefore, buffer capacity of buffer solutions at pH value 4, 7 and 9 is not sufficient to change the pH from highly acidic pH 0. Addition of sodium hydroxide solution did not change pH value of test item in buffer solution and the pH remained 0. Therefore, the effect of pH could not be investigated as this was not technically feasible and not required as the pKa was outside the environmental range (please refer to section 3.6 of the study report). The water solubility of N-MIT·HCl is very high at 420.3 g/L, Increasing or decreasing the temperature would not have significant effect on the water solubility. The substance would still be highly soluble in water, and therefore it is not deemed necessary to investigate the effect of temperature.		Not applicable
Partition coefficient (n-octanol/water) and its pH dependency	log P_{ow}= - 0.44 at 20 °C pH = ca. 2 (shake flask method)	OECD Guideline 107 EU Method A.8 EPA OPPTS 830.7550	The test item is acidic and has a pKa of 2.513. It is not technically feasible to investigate the effect of the pH as the buffering capacity is insufficient. Also, the investigation of pH is not required as the pKa is outside the environmental range. Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Wöhr, 2018

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Thermal stability and identity of breakdown products	The active ingredient (MIT) is stable in air under both sets of criteria N-MIT·HCl: Not applicable	Two methods were used, both acceptable under the guidelines of OECD 113	Not applicable	Cihiy, 1995 (LoA)
		Waiver: study scientifically not necessary / other information available Laboratories are unable to investigate the breakdown products of the test item. The boiling point and melting point are already provided in relevant sections. Running of the OECD 113 test would not generate any additional data than that already generated and supplied, the boiling point and melting point, therefore it is not necessary to run any additional testing. Furthermore, the thermal stability study OECD 113 BPR guidance states thermal breakdown products are to be identified if possible. The test item melted in the range of 165.0 °C to 170.0°C (438.2 K to 443.2 K). The atmospheric pressure was 99.04 kPa. The test item had no boiling point according to the criteria described in the guideline with an observation up to 400 °C. The atmospheric pressure was 99.04 kPa.		Not applicable
Reactivity towards container material	Not applicable	Not applicable	No signs of reactivity with container material from the history of use. N-MIT·HCl is packaged in HDPE plastic white containers/bottles with a cap. This packaging has been used for over 2 years, with no complaints received from customers regarding the package integrity. The packaging characteristics will be monitored further during the ambient storage stability, which is	Not applicable

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			a data requirement for product authorisation.	
Dissociation constant	pKa = 2.513 at 20°C (titration with 0.05 M NaOH)	OECD Guideline 112 EPA OPPTS 830.7370	The titration method was performed in accordance with the guideline at 0.01 mol/L in pure water. Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	Fieseler, 2017b
Viscosity	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid [study technically not feasible].	Not applicable
Solubility in organic solvents, including effect of temperature on solubility	1) Stable concentrations: ethyl acetate: 315.4 mg/L at 10°C, 446.1 mg/L at 20°C, 545.9 mg/L at 30°C n-hexane: 3.4 mg/L at 10°C, 7.7 mg/L at 20°C, 16.5 mg/L at 30°C 2) MIT: There is a significant effect on solubility on increasing the temperature from 10ºC to 30ºC, for both ethyl acetate and hexane	1) CIPAC MT 181 OECD Guideline 105 2) OECD Guideline 116	Shake flask method. Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 %	1) Wöhr, 2020 2) Betteley, 2001 (LoA)
Stability in organic solvents used in biocidal products and identity of relevant degradation products	Not applicable	Not applicable	This study was not performed because the product, as manufactured, does not include an organic solvent. It is a powder.	Not applicable

A.1.4. Physical hazards and respective characteristics

Table A.7 Physical hazards and respective characteristic

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
Explosives	European Regulation (EC) No. 528/2021, European Regulation (EC) No. 440/2008, differential scanning calorimetry (DSC)	Test material: N-MIT·HCl Batch Number 47193000 onset temperature of energetic activity, decomposition energy	Exotherm onset temperature (°C): 157.2 and 328.0 Decomposition energy ($J \cdot g^{-1}$) 389.3 and 44.9. Substance is exempt from classification as a UN Class 1 explosive substance since the total decomposition energy was <500 $J \cdot g^{-1}$ and the onset of exothermic decomposition was <500°C.	Gledhill, 2021
Flammable gases	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Flammable aerosols	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Oxidising gases	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Gases under pressure	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Flammable liquids	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Flammable solids	UN Manual of Tests and Criteria: Test N.1	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 % Burning rate (preliminary screening test)	Substance does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the 2 minutes test period	Wagner, 2017 (UN N1 Test)
Self-reactive substances and mixtures	Heat Accumulation Storage Test (UN Test H.4)	Test material: N-MIT·HCl Batch Number 53712300 Self-accelerating	SADT > 75°C for a 50 kg package The test item is exempt from classification as a self-reactive substance of UN Class 4,	Gledhill, 2022

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
		decomposition temperature (SADT) for a 50 kg package of the test item, N-MIT.HCl.	Division 4.1 since the SADT for a 50 kg package was > 75°C.	
Pyrophoric liquids	Not applicable	Not applicable	The substance is not a pyrophoric liquid as it is a solid.	Not applicable
Pyrophoric solids	Procedure designed to be compatible with Method A13 Pyrophoric Properties of Solids and Liquids of Commission Regulation (EC) No 440/2008 of 30 May 2008	Not applicable	No pyrophoric properties. Based on experience in handling and use of the test item during testing, the result of the pyrophoric properties test has been predicted negative. Additionally, the DSC under 30 bar air shows no exotherm up to 100°C.	Physchem waivers & Roche document BS-9626
Self-heating substances and mixtures	UN Test N.4, 2009	Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9 % relative self-ignition temperature	In conclusion, a negative result was obtained with the test item N-MIT·HCl in 25 mm (at 140 °C and at 100 °C) and 100 mm cube (at 100 °C) samples. The test item N-MIT·HCl is not a self-heating substance.	Rivas V.W., 2017
Substances and mixtures which in contact with water emit flammable gases	Procedure designed to be compatible with method EC A.12 flammability (contact with water) of solids and liquids	Not applicable	Based on experience on handling and use of the test item during testing, the results of the flammability (contact with water) test have been predicted negative.	Physchem waivers & Roche document BS-9626
Oxidising liquids	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Oxidising solids	Not applicable	Not applicable	The study does not need to be conducted because there are no chemical groups	BS-9626_EN

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			present in the molecule which are associated with oxidising properties and hence, the classification procedure does not need to be applied. The presence of HCl should have no impact on the oxidising properties of N-MIT as the Cl is bonded to H, therefore the results are predicted negative.	
Organic peroxides	Not applicable	Not applicable	The study does not need to be conducted because the substance does not fall under the definition of organic peroxides according to GHS and the relevant UN Manual of tests and criteria.	Not applicable
Corrosive to metals	Not applicable	Not applicable	The application of the test C.1 (UN Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria) is not applicable as the test material is a solid, it has a high melting point of 165-170°C therefore will not become a liquid in transportation. Testing under C.1 is therefore not deemed necessary.	Physchem waivers, 2018 and BS-9626, 2010
Auto-ignition temperature (liquids and gases)	Not applicable	Not applicable	The study does not need to be conducted because the substance is a solid.	Not applicable
Relative self-ignition temperature for solids	Method 33.3.1.6 "Test N.4: Test method for self-heating substances"	Test in the 25 mm cube at 140°C and at 100°C and in the 100 mm cube at 100°C	No self-ignition observed under the test conditions (>140 °C) Test material: N-MIT·HCl Batch No.: 10013913, Purity 99.9%	Wagner, 2017
Dust explosion hazard	Not applicable	Not applicable	Waiver: Based on the nature of the test item, as it is coarse granular,	Physchem waivers, 2018 &

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			the dust explosion is predicted negative.	document BS-9626, 2010

A.1.5. Assessment of physical hazards according to the CLP criteria

A.1.5.1. Assessment of physical hazards

A.1.5.2. Explosives

Table A.8 Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Differential scanning calorimetry (DSC)	Exotherm onset temperature (°C): 157.2 and 328.0 Decomposition energy ($J \cdot g^{-1}$): 389.3 and 44.9	Not explosive	Gledhill, 2021

A1.5.2.1 Short summary and overall relevance of the provided information on explosive properties

The structure of the 2-methyl-2H-isothiazol-3-one hydrochloride shows that there are no chemical groups associated with explosive substances present.
Examples of groups which may indicate explosive properties in organic materials are:

- C-C unsaturation (e.g. acetylenes, acetylides, 1, 2-dienes);
- C-Metal, N-Metal (e.g. Grignard reagents, organo-lithium compounds);
- Contiguous nitrogen atoms (e.g. azides, aliphatic azo compounds, diazonium salts, hydrazines, sulphonylhydrazides);
- Contiguous oxygen atoms (e.g. peroxides, ozonides);
- N-O (e.g. hydroxyl amines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles);
- N-halogen (e.g. chloramines, fluoroamines);
- O-halogen (e.g. chlorates, perchlorates, iodosyl compounds)

The composition does contain N, O and Cl and therefore a DSC screening test was performed.
The DSC screening tested resulted in a total decomposition energy of <500 J g^{-1} and the onset of exothermic decomposition was <500°C.

A1.5.2.2 Comparison with the CLP criteria

According to part 2, 2.1.4.3 of Annex I of CLP Regulation, 2-methyl-2H-isothiazol-3-one hydrochloride shall not be classified as explosive considering that are no chemical groups associated with explosive properties in the molecule as well as the results of DSC screening test.

A1.5.2.3 Conclusion on classification and labelling for explosive properties

No classification is proposed for 2-methyl-2H-isothiazol-3-one hydrochloride regarding explosives hazards according to CLP criteria.

A.1.5.3. Flammable gases (including chemically unstable gases)

Not applicable for CLH report.

A.1.5.4. Flammable aerosols and aerosols

Not applicable for CLH report.

A.1.5.5. Oxidising gases

Not applicable for CLH report.

A.1.5.6. Gases under pressure

Not applicable for CLH report.

A.1.5.7. Flammable liquids

Not applicable for CLH report.

A.1.5.8. Flammable solids

Table A.9 Summary table of studies on flammable solids

Method	Results	Remarks	Reference
UN Manual of Tests and Criteria: Test N.1 (Test method for readily combustible solids); burning rate test: preliminary screening test	Substance does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the 2 minutes test period	The test item melted in the range between 165.0°C to 170.0°C.	Wagner, 2017 (UN N1 Test)

A1.5.8.1 Short summary and overall relevance of the provided information on flammable solids

The test item N-MIT·HCl was used in its initial form during the test UN Manual of Tests and Criteria: Test N.1 (Test method for readily combustible solids). The test item melted in the burning flame, according to study the test item melted in the range between 165.0°C to 170.0°C. The test item could not be ignited, no smouldering was observed and at the end of the measurement the test item was black.

A1.5.8.2 Comparison with the CLP criteria

According to part 2, 2.7.2.3 of Annex I of CLP Regulation, N-MIT·HCl shall not be classified as flammable considering the negative results of screening test. The test item melted when exposed to the burning flame and therefore did not ignite or propagate combustion, the burn time is > 2 mins and therefore no burning rate could be calculated.

A1.5.8.3 Conclusion on classification and labelling for flammable solids

No classification is proposed for N-MIT·HCl regarding flammable solids hazards according to CLP criteria.

A.1.5.9. Self-reactive substances

Table A.10 Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
UN Manual of Tests and Criteria: (UN H.4) Heat Accumulation Storage Test	SADT > 75°C for a 50 kg package	Not self-reactive	Gledhill, 2022

A1.5.9.1 Short summary and overall relevance of the provided information on self-reactive substances

The test item N-MIT·HCl 2 was used in its initial form during the test UN Manual of Tests and Criteria: Test H.4 (Heat accumulation storage test). The test item was observed to take 37.9 hours to reach a temperature 2°C below the oven temperature. Over the following 168 hours (7 days) the test item reached a maximum temperature of 75.3°C.

A1.5.9.2 Comparison with the CLP criteria

According to part 2, 2.8.2.3 of Annex I of CLP Regulation, 2-methyl-2H-isothiazol-3-one hydrochloride shall not be classified as self-reactive substances as ithe did not reach a temperature of more than 6°C above the oven temperature therefore the SADT of the test item is > 75°C.

A1.5.9.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed for N-MIT·HCl regarding self-reactive substances hazards according to CLP criteria.

A.1.5.10. Pyrophoric liquids

Not applicable for CLH report.

A.1.5.11. Pyrophoric solids

Table A.11 Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Not applicable	Not applicable	Waiver: Based on experience in handling and use of the test item during testing, the result of the pyrophoric properties test has been predicted negative. Additionally, the DSC under 30 bar air shows no exotherm up to 100°C.	Physchem waivers, 2018 & document BS-9626, 2010

A1.5.11.1 Short summary and overall relevance of the provided information on pyrophoric solids

Based on experience in handling and use of the test item during testing, the result of the pyrophoric properties test has been predicted negative, using a procedure designed to be compatible with Method A13 Pyrophoric Properties of Solids and Liquids of Commission Regulation (EC) No 440/2008 of 30 May 2008. Additionally,

the DSC under 30 bar air shows no exotherm up to 100°C.

A1.5.11.2 Comparison with the CLP criteria

According to part 2, 2.10.2 of Annex I of CLP Regulation, 2-methyl-2H-isothiazol-3-one hydrochloride shall not be classified as pyrophoric solids as the DSC under 30 bar air shows no exotherm up to 100°C.

A1.5.11.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed for N-MIT·HCl regarding pyrophoric solids hazards according to CLP criteria .

A.1.5.12. Self-heating substances

Table A.12 Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
UN Test N.4, 2009	A negative result was obtained with the test item N-MIT·HCl in 25 mm (at 140 °C and at 100 °C) and 100 mm cube (at 100 °C) samples. The test item N-MIT·HCl is not a self-heating substance.	Determination of the relative self-ignition temperature	Rivas V.W., 2017

A1.5.12.1 Short summary and overall relevance of the provided information on self-heating substances

The relative self-ignition temperature was determined according to the test method UN Test N.4 (2009). In conclusion, a negative result was obtained with the test item N-MIT·HCl in using 25 mm (at 140°C and at 100°C) and 100 mm cube (at 100°C) samples.

A1.5.12.2 Comparison with the CLP criteria

According to part 2, 2.11.2 of Annex I of CLP Regulation, 2-methyl-2H-isothiazol-3-one hydrochloride shall not be classified as self-heating substance as a negative result was obtained in using 25 mm (at 140°C and at 100°C) and 100 mm cube (at 100°C) samples.

A1.5.12.3 Conclusion on classification and labelling for self-heating substances.

No classification is proposed for N-MIT·HCl regarding self-heating substance hazards according to CLP criteria.

A.1.5.13. Substances which in contact with water emit flammable gases

Table A.13 Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Not applicable	Not applicable	Waiver: N-MIT·HCl does not decompose with water giving flammable gases.	Physchem waivers, 2018 & document BS-9626, 2010

A1.5.13.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Based on experience on handling and use of the test item during testing, the results of the flammability (contact with water) test have been predicted negative, using a procedure designed to be compatible with method EC A.12 flammability (contact with water) of solids and liquids. Please refer to the supporting documents attached.

A1.5.13.2 Comparison with the CLP criteria

According to part 2, 2.12.2 of Annex I of CLP Regulation, 2-methyl-2H-isothiazol-3-one hydrochloride shall not be classified as self-heating substance as it does not decompose with water giving flammable gases.

A1.5.13.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed for N-MIT·HCl according to the CLP criteria for substances which in contact with water emit flammable gases.

A.1.5.14. Oxidising liquids

Not applicable for CLH report.

A.1.5.15. Oxidising solids

Table A.14 Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Not applicable	Not applicable	The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with oxidising properties and hence, the classification procedure does not need to be applied. The presence of HCl should have no impact on the oxidising properties of N-MIT as the Cl is bonded to H, therefore the results are predicted negative.	BS-9626_EN

A1.5.15.1 Short summary and overall relevance of the provided information on oxidising solids

No chemical groups present in the molecule which are associated with oxidising properties. The presence of HCl should have no impact on the oxidising properties of N-MIT·HCl as the Cl is bonded to H, therefore the results are predicted negative.

A1.5.15.2 Comparison with the CLP criteria

Not applicable as no chemical groups present in the molecule which are associated with oxidising properties. Hence, the classification procedure regarding CLP does not need to be applied.

A1.5.15.3 Conclusion on classification and labelling for oxidising solids

N-MIT·HCl is not classified as oxidising solids.

A.1.5.16. Organic peroxides

Table A.15 Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
Not applicable	Not applicable	The study does not need to be conducted because the substance does not fall under the definition of organic peroxides according to CLP Regulation and the relevant UN Manual of tests	Not applicable

A1.5.16.1 Short summary and overall relevance of the provided information on organic peroxides

N-MIT·HCl does not fall under the definition of organic peroxides according to CLP criteria and the relevant UN Manual of tests

A1.5.16.2 Comparison with the CLP criteria

Not applicable as N-MIT·HCl does not fall under the definition of organic peroxides according to part 2, 2.15.1 of Annex I of CLP Regulation.

A1.5.16.3 Conclusion on classification and labelling for organic peroxides

N-MIT·HCl is not classified as organic peroxides.

A.1.5.17. Corrosive to metals

Table A.16 Summary table of studies on the hazard class corrosive to metals*

Method	Results	Remarks	Reference
Not applicable	Not applicable	Waiver: N-MIT·HCl is classified as corrosive to skin, therefore would usually be classified as H290, however the application of the test C.1 (UN Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria) is not applicable as the test material is a solid, it has a high melting point of 165-170°C therefore will not become a liquid in transportation. Testing under C.1 is therefore not deemed necessary and no classification is required.	Physchem waivers, 2018 and BS-9626, 2010

A1.5.17.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

2-methyl-2H-isothiazol-3-one hydrochloride is classified as corrosive to skin (Anonymous 1, 2017), therefore would usually be classified as H290, however the application of the test C.1 (UN Recommendations on the Transport of Dangerous Goods Manual of Tests and Criteria) is not applicable as the test material is a solid, it has a high melting point of 165-170°C therefore will not become a liquid in transportation. Testing under C.1 is therefore not deemed necessary.

A1.5.17.2 Comparison with the CLP criteria

Not applicable as N-MIT·HCl is a solid, it has a high melting point of 165-170°C therefore will not become a liquid in transportation. Therefore testing under C.1 is not deemed necessary as well so no classification according to the criteria in part 2, 2.16.4.1 of Annex I of CLP Regulatin is required.

A1.5.17.3 Conclusion on classification and labelling for corrosive to metals

N-MIT·HCl is not classified as corrosive to metals.

A.1.6. Analytical methods for detection and identification

Not applicable for the CLH report.

A.2. Effects against target organisms

The active substance N-MIT·HCl is a broad spectrum antimicrobial substance and it is intended to be used as a preservative in aqueous solutions in life science, specifically in reagents for scientific research and development in life science sector and in the production of biomolecules. N-MIT·HCl is intended to prevent the biodeterioration of the material and is active against bacteria, yeast and fungi.

A.2.1. Intended uses

Not applicable for the CLH report.

A.2.2. Summary on efficacy

A.2.2.1. Efficacy

Not applicable for the CLH report.

A.2.2.2. Mode of action

Pure/non aqueous N-MIT·HCl is only present in solid state immediately after manufacturing. Once N-MIT·HCl is in an aqueous solution, it dissociates into N-MIT and HCl. Chloride and hydrogen ions originating from N-MIT·HCl do not represent a biologically significant amount and do not contribute to the overall efficacy at the used N-MIT·HCl concentrations. N-MIT in aqueous solution originating from N-MIT·HCl is chemically indistinguishable from N-MIT in aqueous solution originating from N-MIT. In an aqueous environment the nitrogen atom in N-MIT can exist as a protonated ion (conjugated acid) or an unprotonated free base with hydrogen from surrounding water molecules being used as the source of protons. The extent of protonation depends on the pH of the local environment. The pH in solutions, where N-MIT is active, is from 6-8. The dominant form for N-MIT will be in unprotonated form at pH 6-8 and thus chemically equivalent to N-MIT. Furthermore, under typical use conditions, in which the active substance is highly diluted (e.g. 0.1% w/w) the protonated form of the active substance will not depend on whether N-MIT·HCl or N-MIT (freebase) was used as biocidal product.

N-MIT utilizes a two-step mechanism involving rapid inhibition (within minutes) of growth and metabolism, followed by an irreversible cell damage resulting in loss of viability (within hours). Cells are inhibited by disruption of the metabolic pathways involving dehydrogenase enzymes. In particular, N-MIT attacks the central metabolic cycle of the cell, the Krebs cycle, at two specific enzymatic sites: succinate dehydrogenase and NADH (Nicotinamide adenine dinucleotide) dehydrogenase. Other inhibited enzymes include: pyruvate dehydrogenase, which is vital for oxidation of pyruvate to acetyl-CoA (Coenzyme A), lactate dehydrogenase and alcohol dehydrogenase, which detoxify waste by-products synthesized during fermentation. Critical physiological functions are rapidly inhibited in microbes, including growth, respiration (oxygen consumption), and energy generation (ATP synthesis). Cell death results from the destruction of protein thiols and the production of free radicals. The rate and extent of killing may be enhanced by various adjuvants including surfactants. This unique mechanism results in a broad spectrum of activity, low use levels, and difficulty in attaining resistance. Cell death results from the destruction of protein thiols and the production of free radicals.

A.3. Assessment of effects on Human Health

N-MIT·HCl will dissociate in contact with water and become (de-) protonated according to the pH of the solution. The pH in solutions, where N-MIT is active, is from 6-8. The dominant form for N-MIT will be unprotonated form at pH 6-8 and thus chemically equivalent to N-MIT. Furthermore, under typical use conditions, in which the active substance is highly diluted (e.g. maximum in-use concentration: 0.999% w/w) the protonated form of the active substance will not depend on whether N-MIT·HCl or N-MIT (free base) was used as biocidal product. Consequently, a comprehensive read-across from the latter to the former is fully justified. The (pure/non aqueous) target is only present in solid state immediately after manufacturing and before entering the major use stages. The acute toxicity of the solid target compound is strongly dominated by high acidity and corrosivity leading to localised toxicity. These effects are also observed for the source compound. Both the target and source are considered to be similarly classified for acute oral toxicity and skin corrosivity (when test concentration is accounted for) with available bacterial gene mutation data on both the target and source confirming a lack of bacterial gene mutation potential. A comparison of the harmonised classification for HCl shows that it presents a similar acute toxicity profile to the target and source compounds following exposure to solids and high strength formulations. Furthermore, the contribution of HCl is already accounted for in the acute oral toxicity and skin corrosivity studies conducted with N-MIT·HCl.

Therefore, it is considered acceptable to read-across from the source for mammalian systemic toxicity, genotoxicity and ecotoxicology endpoints since in all cases aqueous media were used as the dose vehicle. The read across has been undertaken using the ECHA Read-Across Assessment Framework (RAAF, 2017a) from the already submitted and approved N-MIT (free base) to the new active substance N-MIT·HCl. Please refer to Document with a justification for the read across available in confidential Annex II (Appendix VIII: Read across between N-MIT·HCl and N-MIT).

Read-across is not proposed for the acute dermal toxicity and acute inhalation toxicity endpoints. Waivers are proposed for these endpoints, based on the demonstrated corrosivity of N-MIT·HCl; acute toxicity testing is not required for corrosive substances. Although read-across data are available for N-MIT, data for N-MIT·HCl indicate a lower pH and also show a more severe classification for skin corrosivity compared to N-MIT. As acute dermal toxicity and acute inhalation toxicity are strongly influenced by site of contact effects that may be more severe for N-MIT·HCl, a waiver is more appropriate for these endpoints.

A.3.1. Toxicokinetics

Read-across is proposed for a number of endpoints using data generated using the source substance N-MIT. As the target substance N-MIT·HCl dissociates in aqueous conditions (i.e. following dosing in studies *in vitro* or *in vivo*, exposure in experimental systems will be to N-MIT, regardless of the test material. Additional exposure to HCl resulting from the dissociation of N-MIT·HCl may result in more severe local effects at high concentrations; however, both substances are indicated as corrosive to skin. An effect of HCl on systemic toxicity is not predicted as the hydrogen and chloride ions are physiologically ubiquitous and subject to homeostatic control. Furthermore, the dose levels used in studies with N-MIT are limited by site of contact effects, which may be more severe for N-MIT·HCl. The proposed read-across therefore represents a robust and scientifically justified approach.

A detailed read-across justification is presented in a separate document (Anonymous, 2021).

Table A.17 Summary table of toxicokinetic studies

Summary table of toxicokinetic studies					
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
OECD draft TG for dermal absorption, GLP, Reliability: 1	Rat (*in vitro*)	*^{14}C-RH-573* (N-MIT 99.88% radiochemical purity), 27, 75, 150 ppm (equiv. 0.66, 1.97, 3.97 µg/cm^2)	36% of the applied dose was absorbed across the skin barrier following a 24 h exposure period	N-MIT	Anonymous 18, 2003 (LoA)
OECD 428, GLP, Reliability: 1	Human epidermis	*^{14}C-RH-573* (N-MIT 96.90% radiochemical purity), 52.2, 104.3 and 313 µg N-MIT/mL 100 µg/mL in CTAE shampoo formulation, body lotion formulation, facial cream	N-MIT in aqueous solution was readily absorbed across the human epidermis following a 24 h occluded exposure – 29.8, 38.0 and 54.7% of applied dose at N-MIT concentrations of 52.2, 104 and 313 µg/mL, respectively. When N-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. The amount of N-MIT (as 14C-label equivalents), when formulated in personal care formulations, absorbed across human skin in 24 h period (systemically available) was 19% of the administered dose	N-MIT	Anonymous 19, 2005 (LoA)

Non-guideline study	Mice, CD-1, ♂/♀, 15/sex	*^{14}C-RH-573* (N-MIT 96.90% radiochemical purity), 100 mg/kg	Radioactivity associated with N-MIT reaches the bone marrow and remains there for up to 48 h.	N-MIT	Anonymous 15, 2003(LoA)
OECD 417, GLP, Reliability: 1	Rat, SD, ♂/♀ 3-4/gp	*^{14}C-RH-573* (N-MIT 96.90% radiochemical purity), 5, 50 mg/kg	Rapid excretion in the urine and faeces; low levels of radioactivity identified in the adrenals, thyroids, ovaries and testes	N-MIT	Anonymous 16, 2005 (LoA)
OECD 417 (bile cannulated), GLP, Reliability: 1	Rat, SD, F 4/gp	*^{14}C-RH-573* (N-MIT 96.90% radiochemical purity), 50 mg/kg	N-MIT was extensively metabolized and excreted primarily into the urine. 53% of N-MIT was absorbed	N-MIT	Anonymous 17, 2005 (LoA)

A3.1.1 Short summary and overall relevance of the provided toxicokinetic information

The absorption of N-MIT from rats treated with 5-50 mg base-eq. N-MIT /kg bw was 92-96%. Absorption and excretion were rapid, with 80-87% of ^{14}C label excreted in 24 hours. N-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 N-MIT in the animal body. N-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, faeces or bile.

In the second toxicokinetic study of N-MIT similar results were obtained after single oral dose 50 mg (^{14}C)-N-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 N-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. N-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 N-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 N-MIT was absorbed. In rats treated with 5 mg/kg bw 67-69% of N-MIT was absorbed. For

the risk assessment 53% will be used as a value for the oral absorption of N-MIT, representing the worst case.

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

When dermal absorption of N-MIT was tested through human skin 65.5, 62.0 and 67.3% of the applied dose was 'potentially' systemically available at N-MIT concentrations of 52.2, 104 and 313 µg/mL (in water), respectively. When N-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 N-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 N-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.

A3.1.2 Values and conclusions used for the risk assessment

Not applicable for the CLH report.

A.3.2. Acute toxicity / STOT SE

A.3.2.1. Acute oral toxicity

Table A.18 Summary table of animal studies on acute oral toxicity

Summary table of animal studies on acute oral toxicity						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other)	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	Reference
Acute oral, up down method, OECD 425 (2008), GLP, Reliability: 1 Key study	Rat, Wistar, Female 1 (55 mg/kg bw), 3 (175 mg/ kg bw), 2 (550 mg/kg bw)	N-MIT·HCl 98.7% Purified water 55, 175, 550 mg/kg bw Gavage	**55 mg/kg bw**: 1 animal tested, no clinical signs **175 mg/kg bw**: 3 animals tested Piloerection: 2/3 Dyspnoea: 2/3 Hunched posture: 2/3 Ataxia: 2/3 Death: 1/3 **550 mg/kg bw:** 2 animals tested Piloerection: 1/2 Decreased activity: 1/2 Dyspnoea: 2/2 Prone posture: 2/2 Hypothermia: 2/2 Ptosis: 2/2 Tonic convulsion: 2/2 Death: 2/2 Humanely killed 4 hours after dosing)	LD_{50} = 175 mg/kg bw	N-MIT·HCl	Anonymous 10, 2018 (unpublished report no.: 8373386)

No human data are available.

No other studies are available.

A3.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The method used to investigate the acute oral toxicity of N-MIT·HCl was assessed the up and down procedure (OECD 425). Study data are presented in Table A.17 (Anonymous 10, 2018). The study design provides information for hazard assessment and classification and enables a chemical to be assigned to toxicity classes but severely restricts animal usage. Individual female rats were dosed sequentially at the following dose levels until one of the stopping criteria (as defined in OECD 425) was met: 175, 550, 175, 550, 175 and 55 mg/kg bw. The test article was dispersed in purified water and administered at a dose volume of 10 mL/kg. Surviving animals were killed on Day 15 and all animals subsequently underwent a full necropsy. Both animals dosed at 550 mg/kg bw were killed *in extremis* within 1 to 4 hours of dosing. One animal dosed at 175 mg/kg bw was found dead on Day 2. Principal signs of reaction to treatment were piloerection and dyspnoea. Less common signs were hunched posture, ataxia, hypothermia and tonic convulsions. These signs developed immediately after dosing and lasted up to 4 hours post dosing. All surviving rats achieved body weight gains during the first and second weeks of the study. No macroscopic changes were noted at necropsy, except for pale lungs, red and thick fundus region of the stomach, gelatinous appearance of the mucosal surface of the fundus region of the stomach and small caecum which were noted in the animal dosed at 550 mg/kg bw that was killed *in extremis* 4 hours after dosing. These macroscopic changes are commonly observed following the oral administration of an unpalatable or slightly irritant test article, with regurgitation and aspiration into pulmonary system and isolated are considered not to associate with systemic toxicity. Under the conditions of this study the rat acute oral LD_{50} was 175 mg/kg bw in female rats.

Detailed data on the clinical signs and mortality following treatment are described as the number of animals which died/number of animals with clinical signs/number of animals used (in parenthesis), and as clinical signs occurring at time after dosing and the duration. At dose level 55 mg/kg bw there were no clinical signs seen (0/1/1). At dose level 175 mg/kg bw there were clinical signs observed in two animals (1/2/3); in first animal, piloerection was noted at 1-4 hours after dosing, and dyspnoea at 2-3 hours after dosing; in second animal there were no clinical signs, and the third animal showed signs of piloerection, dyspnoea, and hunched posture at 2-4 hours after dosing, and ataxia at 3-4 hours after dosing, and was found dead the second day of dosing. At dose level 550 mg/kg bw (2/2/2), the first animal displayed piloerection at 1-6.5 hours after dosing, a decreased activity at 2-4 hours, dyspnoea at 2-6.5 hours after dosing, and signs of ptosis, prone posture, and hypothermia at 6.5 hours after dosing when it was humanely killed; the second animal showed signs of prone posture and ptosis immediately after dosing, dyspnoea at 15 minutes, and hypothermia and tonic convulsions 1 hour after dosing when it was humanely killed.

A3.2.1.2 Comparison with the CLP criteria

The acute oral LD_{50} of N-MIT·HCl was 175 mg/kg bw in female rats. Therefore, according to Annex I for Regulation (EC) 1272/2008

N-MIT·HCl must be classified in Category 3 in respect of its acute oral toxicity. The signal word 'Danger' and hazard statement H301 'Toxic if swallowed' is required.

A3.2.1.3 Conclusion on classification and labelling for acute oral toxicity

In accordance with Annex I for Regulation (EC) 1272/2008, N-MIT·HCl is deemed to be toxic by the oral route. Consequently, classification for this route is required:

Oral: Acute Tox. 3; H301 'Toxic if swallowed'; ATE = 175 mg/kg bw

A3.2.1.4 Conclusion on acute oral toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.2. Acute dermal toxicity

Table A.18 Summary table of animal studies on acute dermal toxicity

Summary table of animal studies on acute dermal toxicity						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels, Surface area,	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	Reference
OECD 402, acute dermal, GLP, Reliability: 1 Key study	Rat, Crl:CD BR, ♂/♀, 6/sex	[Read-across] Kordek™ 573T (53.2% N-MIT) 100, 200, 400 mg/kg bw N-MIT (♂/♀), 300 mg/kg (M)	**100 mg/kg bw**: no deaths **200 mg/kg bw**: 2/6 females were found dead at 24 hours; 1 female was found dead at 48 hours **300 mg/kg bw**: 3/6 males were found dead at 24 hours; 2 males were found dead at 48 hours **400 mg/kg bw**: 5/6 males and 5/6 females	242 mg/kg bw N-MIT	Study performed with N-MIT	Anonymous 14, 1999 (LoA)

			were found dead at 24 hours; one female was found dead at 48 hours. Clinical signs were noted beginning on day 1 and included: red material around muzzle, red material around eyes, scant and/or no faeces, passiveness, laboured breathing, and ataxia. Surviving rats recovered from these signs by day 5. Body weight gain in surviving rats was decreased (29-48%) among both sexes at 200 mg/kg bw and above when compared to historical control values. Skin effects were observed in both sexes at all levels beginning on Day 1 and continuing to Day 14. These effects included blanching, edema, darkened areas, eschar, sloughing, scabbed areas and desiccation.			
OECD 402, acute dermal, GLP	Rat (Wistar), 5/sex/ group	Acticide SR 3267 (49.0% N-MIT) 4082 mg/kg bw (2000 mg/kg bw N-MIT)	**2000 mg/kg bw:** severe erythema, very slight/ slight oedema, scabbing, eschar (5M, 4F); body weight stasis (F)	>2000 mg/kg bw N-MIT	Study performed with N-MIT	Anonymous 23, 2000 (in CLH Report for MIT, 2015)

No human data are available.

No other studies are available.

A3.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No data are available for N-MIT·HCl. The biocidal active substance dossier does not contain any data for this endpoint; a waiver was proposed on the basis of corrosivity, in line with the BPR data requirements. However, following the accordance check and in response to a request from ECHA, the data available for N-MIT are included here. It should be noted, however, that differences in pH between N-MIT and N-MIT·HCl (which is more acidic) may contribute to the severity of local effects, and hence also to greater acute dermal toxicity. Consequently, read-across from the study with N-MIT for this endpoint may underestimate acute dermal toxicity and may not therefore not be appropriate.

In an acute dermal toxicity study performed with N-MIT (summarized data in Table A.18, Anonymous 14, 1999), mortality was reported at dose levels of 200 mg/kg bw (0/6M, 3/6F), 300 mg/kg bw (6M) and 400 mg/kg bw (5M, 6F). No deaths were reported at 100 mg/kg bw. Clinical observations were performed at 1, 2 and 4 h after dosing and daily thereafter for 14 days. Clinical signs were noted beginning on day 1 and included: red material around muzzle, red material around eyes, scant and/or no faeces, passiveness, laboured breathing, and ataxia. Surviving rats recovered from these signs by day 5. Body weight gain in surviving rats was decreased (29-48%) among both sexes at 200 mg/kg bw and above when compared to historical control values. Skin effects were observed in both sexes at all levels beginning on Day 1 and continuing to Day 14. These effects included blanching, edema, darkened areas, eschar, sloughing, scabbed areas and desiccation. An acute dermal LD_{50} of 242 mg/kg bw N-MIT was calculated on the basis of the results of this study.
Clinical signs related to possible narcotic effects were passiveness and ataxia. Passiveness was not recorded in male animals at 100 mg/kg, but recorded in 2/6 (two out of six, 33%) males at 200 mg/kg 1 hour after dosing, 3/6 (50%) males at 300 mg/kg 1 hour after dosing, and 1/6 males (17%) at 400 mg/kg 1 and 2 hours after dosing; in 2/6 females (33%) at 100 mg/kg 1 hour after dosing, 4/6 (67%) females at 200 mg/kg 1 hour after dosing, and 1/6 (17%) females at 400 mg/kg 1 hour after dosing); ataxia was not recorded in male animals at 100 and 200 mg/kg, but recorded in 1/6 males (17%) at 300 and 400 mg/kg at 1 hour after dosing; not recorded in female animals at 100 mg/kg, recorded in 3/6 females (50%) at 200 mg/kg 1 hour after dosing, and 1/6 females (17%) at 400 mg/kg 1 hour after dosing. Summary below shows percentage of animals affected:

Dosage, mg/kg bw				
	100	200	300	400
Passiveness				
male	0%	33%	50%	17%
female	33%	67%	not tested	17%
Ataxia				
male	0%	0%	17%	17%
female	0%	50%	not tested	17%

In a further acute dermal toxicity study performed with N-MIT summarised in the CLH Report for MIT, 2015 (summarized data in Table A.18, Anonymous 23, 2000), no mortality was reported at the single dose level of 2000 mg/kg bw. No clinical signs of intoxication were observed in this study. Strong irritation of skin was observed and no systemic toxicity. Signs of local irritation (severe erythema, very slight or slight oedema, scabbing and/or eschar) were reported for all males and four of five females. Males gained weight over the study period; however, body weight stasis was apparent in females. The acute dermal LD_{50} of N-MIT was therefore found to be >2000 mg/kg bw under the conditions of this study.

A3.2.2.2 Comparison with the CLP criteria

The rat acute dermal LD_{50} was 242 mg/kg bw in male and female rats in one study, and >2000 mg/kg bw in another study. Therefore, according to Annex I of Regulation (EC) 1272/2008 N-MIT is classified in Category 3 in respect of its acute dermal toxicity. The signal word 'Danger' and hazard statement H311 'Toxic in contact with skin' is required. However, as noted above, the relevance of the study with N-MIT to N-MIT·HCl is questionable.

A3.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

The results of acute dermal toxicity studies in the rat with N-MIT support classification according to Regulation (EC) 1272/2008 in Acute Tox Cat 3 for this substance. Nevertheless, the relevance of this study to N-MIT·HCl is unclear due to differences in pH and consequently also differences in local toxicity, which may contribute to acute dermal toxicity. Therefore no classification for acute dermal toxicity is proposed for N-MIT·HCl according to Regulation (EC) 1272/2008.

A3.2.2.4 Conclusion on acute dermal toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.3. Acute inhalation toxicity

Table A.19 Summary table of animal studies on acute inhalation toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body/ head only)	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	-
OECD 403, acute inhalation, GLP, Reliability: 1 Key study	Rat, Crl:CD BR, ♂/♀, 6/sex	RH-573 Technical (97.8% MIT), 0.012, 0.046, 0.15, 1.07, 2.09 mg/L air, nose only (vapour, MMAD: 3.1-5.3 µm; GSD: 2.0-2.4; respirable fraction 33.5-54%)	**0.012 mg/L**: no deaths **0.046 mg/L:** 1/6 males died during exposure **0.150 mg/L**: 2/6 males and 4/6 females died during exposure; 1 male and 1 female were found dead at 24 hours **1.07 mg/L**: 6/6 males and 3/6 females died during exposure **2.09 mg/L**: 3/6 males and 1/6 females died during exposure; 2 males and 2 females died at 1 hour post exposure; one male was found dead at 24 hours. Clinical signs seen in some,	0.11 mg/L N-MIT	Study performed with N-MIT	Anonymous 11, 1995 (LoA)

			yet not all groups included gasping, rales, labored breathing, respiratory noise, salivation, red-stained eyes and muzzle, nasal exudate, passiveness, and ataxia. There were no exposure-related effects on body weight			
OECD 403, acute inhalation, GLP, Reliability: 1 Key study	Rat, Crl:CD BR, ♂/♀, 5/sex	Kordek™ 573T (53.2% MIT) 0.15, 0.25, 0.47 and 0.68 mg/L (N-MIT) air, nose only (vapour, MMAD: 2.2-2.5 µm; GSD: 1.7-1.9)	**0.15 mg/L**: no deaths **0.25 mg/L:** 2/5 males and 1/5 females died during exposure; 1 male and a female died on the day following exposure **0.47 mg/L:** 1/5 males and 2/5 females died during exposure; 1 female died on the day following exposure **0.68 mg/L:** 3/5 males and 4/5 females died during exposure; 2 males died on the day following exposure. Clinical signs were noted in both sexes at all exposure levels and included: respiratory noise, gasping, rales, labored breathing, salivation, ataxia/abnormal gait, prostration, passiveness, scant and/or no feces, red material around eyes and/or muzzle, clear discharge	0.19 mg/L N-MIT	Study performed with N-MIT	Anonymous 12, 2001, 2002 (LoA)

			from the nose, wet-matted fur around the muzzle, arched back, and/or unkempt.			
OECD 403, acute inhalation, GLP	Rat, Crl(Wi)BR 5/sex/ group	Acticide SR 3267 (49.8% N-MIT) Nose-only 0, 0.086, 0.173, 0.327 mg/L Acticide SR 3267 0, 0.042, 0.086, 0.163 mg/L N-MIT	**0.327 mg/L:** mortality, (Day 0/1) **0.173 mg/L:** mortality, (Day 0/1); dyspnoea, cyanosis, laboured breathing, hypoactivity, tremor, incoordination, **0.086 mg/L:** hypoactivity. squatting, piloerection. Increased breathing, red ocular/nasal discharge	0.134 mg/L N-MIT	Study performed with N-MIT	Anonymous 31, 2000 (in CLH Report for MIT, 2015)

No human data are available.

No other studies are available.

A3.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

No data are available for N-MIT·HCl. The biocidal active substance dossier does not contain any data for this endpoint; a waiver was proposed on the basis of corrosivity, in line with the BPR data requirements. However, following the accordance check and in response to a request from ECHA, the data available for N-MIT are included here. It should be noted, however, that differences in pH between N-MIT and N-MIT·HCl (which is more acidic) may contribute to the severity of local effects, and hence also to greater acute inhalation toxicity. Consequently, read-across from the study with N-MIT for this endpoint may underestimate acute inhalation toxicity and may not therefore be appropriate.

In a study performed with N-MIT (summarized in Table 19; Anonymous 11, 1995), groups of rats (6/sex) were exposed to N-MIT at concentrations of 0.012, 0.046, 0.15, 1.07, 2.09 mg/L for 4 hours (nose only). There were no deaths in the 0.012 mg/L exposure group. Most of the deaths occurred during the exposure and were considered due to the exposure of test material. Clinical signs seen in some, yet not all groups included gasping, rales, labored breathing, respiratory noise, salivation, red-stained eyes and muzzle, and nasal exudate, passiveness, and ataxia. The later two clinical signs are related to possible narcotic effects, and therefore described in more detail. Ataxia was recorded in 1/6 females (17%) exposed to 2,09 mg/L at 3 hours post exposure. Passiveness was recorded in 4/6 males (67%) exposed to 0,15 mg/L at 3 hours and at first day post exposure. There were no exposure-related effects on body weight gain of survivors. Necropsies

revealed that animals in all the groups (either found dead or surviving) showed signs of slight to severe redness in all lobes of the lung. Scattered incidences of red pinpoint foci on the lungs and gas-filled stomachs were also observed. These necropsy observations were consistent with the clinical signs of respiratory irritation. The combined male and female LC_{50} was calculated to be 0.11 m/L N-MIT (95% confidence limits of 0.07 to 0.25 mg/L).

In an additional study performed with N-MIT (summarized in Table 19, Anonymous 12, 2001), groups of rats (5/sex) were exposed for 4 hours (nose-only) to Kordek 573T (53.2% MIT) at N-MIT concentrations equivalent to 0.15, 0.25, 0.47 and 0.68 mg/L. Deaths occurred at 0.25 mg/L (2M, 3F), 0.47 mg/L (1M, 3F) and 0.68 mg/L (5M, 4F); deaths occurred during exposure or within 24 hours. Numerous clinical signs of toxicity were noted upon removal from the chamber through day 6. These clinical signs were noted in both sexes at all exposure levels and included: respiratory noise, gasping, rales, labored breathing, salivation, ataxia/abnormal gait, prostration, passiveness, scant and/or no feces, red material around eyes and/or muzzle, clear discharge from the nose, wet-matted fur around the muzzle, arched back, and/or unkempt. Clinical signs related to possible narcotic effects where passiveness, salivation, prostration, ataxia/abnormal gait, and arched back. Percentage of animals affected during exposures are presented below, for 5 animals per sex tested. Necropsy of the decedents revealed pale and/or reddened lungs, distended intestines and/or wet muzzle. Necropsy of the survivors revealed no gross changes. Females exposed to 0.25 mg/L and above had reduced (25-39%) body weight gain during the 14-day observation period compared to historical controls. There was no effect on body weight in surviving males during the 14-day observation period. The four-hour LC_{50} for Kordek™ 573F was 0.35 mg/L (95% confidence limits of 0.27-0.45 mg/L) for males and females, equivalent to 0.19 mg/L N-MIT.

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

female	0%	0%	20%	0 hrs		
Ataxia						
male	0%	0%	40% 20% 33% 50%	0 hrs 3.5 hrs 1 day 2 days		
female	0%	0%				
Abnormal gait						
male	0%	0%			20% 50%	3.5 hrs 1 day
female	0%	0%				
Arched back						
male	0%	0%				
female	0%	0%	50%	2-5 days		

In a further study with N-MIT summarised in the CLH Report (summary presented in Table 19, Anonymous 31, 2000, in CLH Report for MIT, 2015), groups of five rats/sex were exposed to Acticide SR 3267 (48.9% N-MIT) at concentrations of 0, 0.086, 0.173 or 0.327 mg/L for 4 hours (nose-only). Mortalities were observed at 0.173 and 0.327 mg/l on the day of exposure and the following day (Day 1). Animals exposed to 0.086 mg/L exhibited slight to moderate activity decrease, squatting position, piloerection, respiration rate increase and reddish discharge around the nose in the first hour after treatment. Animals recovered in the second hour after treatment. At 0.173 mg/L, dyspnoea and laboured breathing occurred in two male rats and one female (second hour of observation). The female animal died (3.5 hour) showing severe dyspnoea and laboured breathing. One male animal was found dead one day after the inhalation exposure. Before dying the animal showed moderate activity decrease, squatting position, cyanosis, piloerection, severe dyspnoea, noisy respiration and reddish discharge around the nose, tremor and incoordination. Survivors showed activity decrease, squatting position, piloerection, incoordination, tremor, dyspnoea, noisy respiration and reddish discharge around the nose from the first hour after the inhalation treatment. Animals recovered between second and three days of observation period. In animals exposed to 0.327 mg/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. The acute inhalation LC_{50} for males was determined to be 0.148 mg MIT/L, and for females was 0.124 mg MIT/L. The results of this study indicate that MIT is respiratory irritant.

A3.2.3.2 Comparison with the CLP criteria

The rat acute inhalation LC_{50} of N-MIT is reported to be 0.11, 0.19 and 0.134 mg/L in male and female rats. Therefore, according to Annex I of Regulation (EC) 1272/2008 N-MIT is classified in Category 2 in respect of its acute inhalation toxicity. The signal word 'Danger' and hazard statement H330 'Fatal if inhaled' is required. However, as noted above, the relevance of the study with N-MIT to N-MIT·HCl is questionable.

A3.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The results of acute inhalation toxicity studies in the rat with N-MIT support classification according to Regulation (EC) 1272/2008 in Acute Tox Cat 2 for this substance. Nevertheless, the relevance of this study to N-MIT·HCl is unclear due to differences in pH and consequently also differences in local toxicity, which may contribute to acute inhalation toxicity. Therefore, no classification for acute inhalation toxicity is proposed for N-MIT·HCl according to Regulation (EC) 1272/2008.

A3.2.3.4 Conclusion on acute inhalation toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.4. Specific target organ toxicity – single exposure Category 1 and 2 (STOT SE 1 and 2)

Table A.20 Summary table of animal studies on Specific Target Organ Toxicity STOT SE 1 and 2

Summary table of animal studies on STOT SE 1 and 2						
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other)	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD_{50}	Remarks (e.g. major deviations)	Reference
Acute oral, up down method, OECD 425 (2008), GLP, Reliability: 1 Key study	Rat, Wistar, Female 1 (55 mg/kg bw), 3	N-MIT·HCl (98.7%) None 55, 175, 550 mg/kg bw Gavage	55 mg/kg bw: none 175 mg/kg bw: Piloerection: 4 h Dyspnoea: 3 h Hunched posture: 3 h Ataxia: 2 h 550 mg/kg bw: Piloerection: 6 h	175 mg/kg bw	N-MIT·HCl	Anonymous 10, 2018 (unpublished report no.: 8373386)

	(175 mg/ kg bw), 2 (550 mg/kg bw)		Dyspnoea: 4 h Prone posture / hypothermia / ptosis / tonic convulsion: immediately prior to death			
OECD 402, acute dermal, GLP, Reliability: 1	Rat, Crl:CD BR, ♂/♀, 6/sex	Kordek™ 573T (53.2% N-MIT) 100, 200, 400 mg/kg N-MIT (♂/♀), 300 mg/kg N-MIT (M)	**100 mg/kg bw**: no deaths **200 mg/kg bw**: 2/6 females were found dead at 24 hours; 1 female was found dead at 48 hours **300 mg/kg bw**: 3/6 males were found dead at 24 hours; 2 males were found dead at 48 hours **400 mg/kg bw**: 5/6 males and 5/6 females were found dead at 24 hours; one female was found dead at 48 hours. Clinical signs were noted beginning on day 1 and included: scant and/or no faeces, passiveness and ataxia. Surviving rats recovered from these signs by day 5. Body weight gain in surviving rats was decreased (29-48%) among both sexes at 200 mg/kg bw and above when compared to historical control values. Skin effects were observed in both sexes at all levels beginning on day 1 and continuing through day 14. These effects included: blanching, edema, darkened areas, eschar, sloughing, scabbed areas and desiccation.	242 mg/kg bw	N-MIT	Anonymous 14, 1999 (LoA)
OECD 402, acute dermal, GLP	Rat (Wistar), 5/sex/ group	Acticide SR 3267 (49.0% N-MIT) 4082 mg/kg bw (2000 mg/kg bw N-MIT)	**2000 mg/kg bw:** severe erythema, very slight/ slight oedema, scabbing, eschar (5M, 4F); body weight stasis (F)	>2000 mg/kg bw N-MIT	N-MIT	Anonymous 23, 2000 (in CLH Report for MIT, 2015)
OECD 403, acute	Rat,	RH-573 Technical	**0.012 mg/L**: no deaths	0.11	N-MIT	Anonymous 11,

inhalation, GLP, Reliability: 1	Crl:CD BR, ♂/♀, 6/sex	(97.8% N-MIT), 0.012, 0.046, 0.15, 1.07, 2.09 mg/L air, nose only (vapour, MMAD: 3.1-5.3 µm; GSD: 2.0-2.4; respirable fraction 33.5-54%)	**0.046 mg/L:** 1/6 males died during exposure **0.150 mg/L**: 2/6 males and 4/6 females died during exposure; 1 male and 1 female were found dead at 24 hours **1.07 mg/L**: 6/6 males and 3/6 females died during exposure **2.09 mg/L**: 3/6 males and 1/6 females died during exposure; 2 males and 2 females died at 1 hour post exposure; one male was found dead at 24 hours. Clinical signs seen in some, yet not all groups included gasping, rales, labored breathing, respiratory noise, salivation, red-stained eyes and muzzle, and nasal exudate. There were no exposure-related effects on body weight	mg/L		1995 (LoA)
OECD 403, acute inhalation, GLP, Reliability: 1	Rat, Crl:CD BR, ♂/♀, 5/sex	Kordek™ 573T (53.2% N-MIT) 1.3, 1.9, 4.6, 5.6 mg/L air, nose only (vapour, MMAD: 2.2-2.5 µm; GSD: 1.7-1.9)	**0.15 mg/L**: no deaths **0.25 mg/L:** 2/5 males and 1/5 females died during exposure; 1 male and a female died on the day following exposure **0.47 mg/L:** 1/5 males and 2/5 females died during exposure; 1 female died on the day following exposure **0.68 mg/L:** 3/5 males and 4/5 females died during exposure; 2 males died on the day following exposure. Clinical signs were noted in both sexes at all exposure levels and included: respiratory noise, gasping, rales, labored breathing, salivation, ataxia/abnormal gait, prostration,	0.35 mg/L (0.19 mg/L N-MIT)	N-MIT	Anonymous 12, 2001, Anonymous 13, 2002 (LoA)

			passiveness, scant and/or no feces, red material around eyes and/or muzzle, clear discharge from the nose, wet-matted fur around the muzzle, arched back, and/or unkempt.			
OECD 403, acute inhalation, GLP	Rat, Crl(Wi)BR 5/sex/ group	Acticide SR 3267 (49.8% N-MIT) Nose-only 0, 0.086, 0.173, 0.327 mg/L Acticide SR 3267 0, 0.042, 0.086, 0.163 mg/L N-MIT	**0.327 mg/L:** mortality, (Day 0/1) **0.173 mg/L:** mortality, (Day 0/1); dyspnoea, cyanosis, laboured breathing, hypoactivity, tremor, incoordination, **0.086 mg/L:** hypoactivity. squatting, piloerection. Increased breathing, red ocular/nasal discharge	0.134 mg/L N-MIT	N-MIT	Anonymous 31, 2000 (in CLH Report for MIT, 2015)

No human data are available.

No other available data.

A3.2.4.1 Short summary and overall relevance of the provided information on STOT SE 1 and 2

Acute oral, dermal and inhalation toxicity studies are available. No additional studies relevant to STOT SE classification are available.

A3.2.4.2 Comparison with the CLP criteria

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 are classified in STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect.

On this basis, classification of N-MIT / N-MIT·HCl with STOT-SE 1 or 2 is not warranted. The acute toxicity studies available for N-MIT and N-MIT·HCl demonstrate acute toxicity, with classification required by all routes of exposure. The acute toxic effects are attributable to local (site of contact) toxicity reflecting the corrosivity of the test material. Classification for STOT SE in Categories 1 and 2 is therefore not relevant, as these effects are covered by the classification of the test material for acute toxicity and corrosivity.

A3.2.4.3 Conclusion on classification and labelling for STOT SE 1 and 2

No classification for STOT SE 1 and 2 is proposed for N-MIT·HCl according to Regulation (EC) 1272/2008.

A.3.2.5. Specific target organ toxicity – single exposure Category 3 (STOT SE 3)

Table A.21 Summary table of animal studies on STOT SE 3

Summary table of animal studies on Specific Target Organ Toxicity STOT SE 3					
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/group	Test substance (including purity), Vehicle, Dose levels, duration of exposure	Results (including type of effect; respiratory tract irritation or narcotic effects)	Remarks (e.g. major deviations)	Reference
OECD 403, acute inhalation, GLP, Reliability: 1	Rat, Crl:CD BR, ♂/♀, 6/sex	RH-573 Technical (97.8% N-MIT 0.012, 0.046, 0.15, 1.07, 2.09 mg/L air, nose only (vapour, MMAD: 3.1-5.3 µm; GSD: 2.0-2.4; respirable fraction 33.5-54%)	**0.012 mg/L**: no deaths **0.046 mg/L:** 1/6 males died during exposure **0.150 mg/L**: 2/6 males and 4/6 females died during exposure; 1 male and 1 female were found dead at 24 hours **1.07 mg/L**: 6/6 males and 3/6 females died during exposure **2.09 mg/L**: 3/6 males and 1/6 females died during exposure; 2 males and 2 females died at 1 hour post exposure; one male was found dead at 24 hours. Clinical signs seen in some, yet not all groups included gasping, rales, labored breathing, respiratory noise, salivation, red-stained eyes and muzzle, and nasal exudate. There were no exposure-related effects on	N-MIT	Anonymous 11, 1995 (LoA)

			body weight		
OECD 403, acute inhalation, GLP, Reliability: 1	Rat, Crl:CD BR, ♂/♀, 5/sex	Kordek™ 573T (53.2% N-MIT) 1.3, 1.9, 4.6, 5.6 mg/L air, nose only (vapour, MMAD: 2.2-2.5 µm; GSD: 1.7-1.9)	**0.15 mg/L**: no deaths **0.25 mg/L:** 2/5 males and 1/5 females died during exposure; 1 male and a female died on the day following exposure **0.47 mg/L:** 1/5 males and 2/5 females died during exposure; 1 female died on the day following exposure **0.68 mg/L:** 3/5 males and 4/5 females died during exposure; 2 males died on the day following exposure. Clinical signs were noted in both sexes at all exposure levels and included: respiratory noise, gasping, rales, labored breathing, salivation, ataxia/abnormal gait, prostration, passiveness, scant and/or no feces, red material around eyes and/or muzzle, clear discharge from the nose, wet-matted fur around the muzzle, arched back, and/or unkempt.	N-MIT	Anonymous 12, 2001, Anonymous 13, 2002 (LoA)
OECD 403, acute inhalation, GLP	Rat, Crl(Wi)BR 5/sex/ group	Acticide SR 3267 (49.8% N-MIT) Nose-only 0, 0.086, 0.173, 0.327 mg/L Acticide SR 3267 0, 0.042, 0.086, 0.163 mg/L N-MIT	**0.327 mg/L:** mortality, (Day 0/1) **0.173 mg/L:** mortality, (Day 0/1); dyspnoea, cyanosis, laboured breathing, hypoactivity, tremor, incoordination, **0.086 mg/L:** hypoactivity.	N-MIT	Anonymous 31, 2000 (in CLH Report for MIT, 2015)

			squatting, piloerection. Increased breathing, red ocular/nasal discharge		

No human data are available.

No other data available.

A3.2.5.1 Short summary and overall relevance of the provided information on STOT SE 3

N-MIT is a corrosive substance and can therefore be considered as respiratory irritant as indicated by the acute inhalation toxicity study in rats, however corrosivity of N-MIT is covered by the skin corrosivity classification, because this is the considered to be the mechanism of pulmonary toxicity. Therefore, N-MIT·HCl, as a corrosive substance is also likely to be a respiratory irritant and should be labelled as EUH071, Corrosive to the respiratory tract.

As N-MIT·HCl is demonstrated to be corrosive to skin with classification in Category 1A, a corrosive effect on the respiratory tract following inhalation exposure is also likely. Acute inhalation toxicity studies with N-MIT demonstrates toxicity associated with clinical signs and histopathological findings consistent with respiratory corrosion/irritation. The N-MIT·HCl and N-MIT are both classified as corrosive to skin; the harmonised classification of N-MIT (Category 1B) further indicates that N-MIT·HCl is also likely to be corrosive to the respiratory tract, if inhaled.

A3.2.5.2 Comparison with the CLP criteria

There are no data from acute toxicity studies that indicate a narcotic effect (e.g. evidence of persistent lethargy, lack of coordination, loss of righting reflex, and ataxia) for N-MIT·HCl, or for N-MIT. The clinical signs reported in acute toxicity studies are consistent with a local irritant effect at the site of contact (and secondary effects) and do not indicate any systemic effect on the nervous system. According to CLP criteria in addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity was corrosivity, the substance or mixture shall also be labelled as 'corrosive to the respiratory tract'. Data for acute inhalation toxicity are not available for N-MIT·HCl, but they are available for N-MIT and confirm the corrosive nature of the compound. EUH071 can also be applied to inhaled corrosive substances not tested for acute inhalation toxicity according to CLP Annex II, Section 1.2.6, therefore, N-MIT·HCl should be labelled as EUH071, Corrosive to the respiratory tract.

A3.2.5.3 Conclusion on classification and labelling for STOT SE 3

According to Regulation (EC) 1272/2008 N-MIT·HCl should be labelled as EUH071, Corrosive to the respiratory tract.

A3.2.5.4 Overall conclusion on acute toxicity related to risk assessment

Not applicable for the CLH report.

A.3.3. Skin corrosion and irritation

Table A.22 Summary table of in vitro studies on skin corrosion/irritation

Summary table of in vitro studies on skin corrosion/irritation					
Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Results	Remarks (e.g. major deviations)	Reference
In vitro EpiDerm skin model OECD 431 (2016) GLP, Reliability: 1 Key study	N-MIT·HCl (98.7%) None 25 mg	Human skin model. Negative control: purified water Positive control: 8N KOH	Skin viability: 3 min exposure: 22% 1 h exposure: 5.6% N-MIT·HCl considered to be corrosive (Sub-category 1A)	N-MIT·HCl	Anonymous 1, 2017 (unpublished report no.: 8366775)

No animal data are available.

No human data are available.

A3.3.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Skin corrosivity

N-MIT·HCl was found to be corrosive to the skin using the three-dimensional human skin model EPIDERM comprising a reconstructed epidermis with a functional stratum corneum (Table A.22, Anonymous 1, 2017)

In a GLP, OECD 431 study, 25 mg of test article or 50 uL of negative or positive control were applied to EpiDerm tissue 3 and 60 minutes followed by a 42 hour post-incubation period and immediate determination of cytotoxic effects via the MTT reduction assay.

Corrosivity potential of the test article was predicted from the relative mean tissue viabilities obtained compared to the corresponding

negative control tissues concurrently treated with distilled water. The positive control, 8N potassium hydroxide viability was <20% following a 60 minute exposure, thereby confirming that corrosivity could be detected in this test system.

The test article showed evidence of corrosivity during both the short term (3 minute) and prolonged exposures (60 minutes). The mean relative tissue viability was <50% (22%) after the 3 minute exposure and <15% (5.6%) after the 60 minute treatment (+ 42 hour post-incubation).

Under the conditions of this study the N-MIT·HCl showed corrosive effects. The mean relative tissue viability was <50% after 3 minute (22%) and 1 hour (5.6%) exposure. Therefore, test article N-MIT·HCl, was considered to be corrosive (Sub-category 1A) according to the UN GHS classification system.

Skin irritation

A waiver for the skin irritation endpoint has been submitted as the available information indicates that the criteria are met for classification as corrosive to the skin.

A 3.3.2 Comparison with the CLP criteria

N-MIT·HCl was deemed to be corrosive to the skin in the EpiDerm *in vitro* corrosivity model conducted under OECD 431. The mean relative tissue viability was <50% (22%) after the 3 minute exposure and <15% (5.6%) after the 60 minute treatment (+ 42 hour post-incubation). In accordance with STEP 2 of the judgement criteria (Table 5, OECD TG 431), as tissue viability was <25% following the 3 minutes exposure, N-MIT·HCl is deemed to be corrosive to skin, Category 1A. Recognising that following the criteria detailed in the test method for assigning classification Sub--Category 1A may result in over-classification in a proportion of cases. Nevertheless, Sub-Category 1A is proposed as a worst-case.

In accordance with Annex I for Regulation (EC) 1272/2008, N-MIT·HCl was deemed to be corrosive to the skin, Sub-Category 1A.

A3.3.3 Conclusion on classification and labelling for skin corrosion/irritation

According to Regulation (EC) 1272/2008 the active ingredient, N-MIT·HCl is deemed to cause skin corrosivity. Consequently, classification for this route is required:

Skin corrosion/irritation Category 1A; H314: 'Causes severe skin burns and eye damage'

A3.3.4 Overall conclusion on skin irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.4. Serious eye damage and Eye irritation

No in vitro data are available

No animal data are available.

No human data are available.

A3.4.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

A waiver for the serious eye damage/eye irritation endpoint has been submitted as the available information indicates that the criteria are met for classification as Eye Dam. 1. Therefore, as N-MIT·HCl is confirmed to cause skin corrosivity, no further testing is required for serious eye damage/eye irritation, with N-MIT·HCl considered corrosive to eyes.

A3.4.2 Comparison with the CLP criteria

Based on the results of the in vitro skin study, N-MIT·HCl showed corrosive effects. The mean relative tissue viability was <50% after 3 minutes (22%) and 1 hour (5.6%) exposure. Therefore, N-MIT·HCl, was considered to be corrosive to skin. Consequently, as N-MIT·HCl is confirmed to cause skin corrosivity, no additional testing is required for ocular irritation/corrosion.

A3.4.3 Conclusion on classification and labelling for serious eye damage/eye irritation

According to Regulation (EC) 1272/2008 the active ingredient, N-MIT·HCl is deemed to cause eye corrosivity. Consequently, classification for this route is required:

Eye Dam.1; H318: 'Causes serious eye damage'

A3.4.4 Overall conclusion on eye irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.5. Skin sensitisation

Table A.19 Summary table of animal studies on skin sensitisation

Summary table of studies relevant for skin sensitisation					
Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Doses	Results	Remarks (e.g. major deviations)	Reference
OECD 429, LLNA, GLP, Reliability: 1	Mice, CBA/J, F, 5/gp	N-MIT (10.37%), water, 0, 1500, 4500, 7600, 13500, 15700, 18000 ppm (measured concentrations of N-MIT),	Stimulation indices (SI): 2.08 at 0.15% N-MIT 2.40 at 0.45% N-MIT 2.23 at 0.76% N-MIT 6.64 at 1.35% N-MIT 4.73 at 1.57% N-MIT 6.62 at 1.8% N-MIT Sensitizer at concentrations greater than 7600 ppm N-MIT (or > 152 µg a.i./cm^2)	N-MIT	Anonymous 5, 2003 (LoA)

Human data report in the following table.

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

Table A.24 Summary table of other studies relevant for skin sensitisation

Summary table of other studies relevant for skin sensitisation					
Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Results	Remarks (e.g. major deviations)	Reference
In silico, OECD (Q)SAR Toolbox v 1.4., non GLP, Supportive study	N-MIT	OECD (Q)SAR Toolbox	N-MIT is predicted to be a skin sensitizer based on protein binding	N-MIT	Anonymous 6, 2017 (unpublished report no.: 0372224-TOX1)

Public domain data taken from CLH report for 2-methylisothiazol-3(2H)-one					
Human patch test, Dept. Of Dermato Allergology, Gentofte Hospital, Denmark, 2010-2012 Supportive study	2766 patients with contact dermatitis Dose: 2000 ppm or 0.2 % N-MIT	-	-2010: 2.0% -2011: 3.0% -2012: 3.7%	N-MIT	**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 Switzerland Supportive study	28,922 dermatitis patients Dose: 500 ppm or 0.05 % N-MIT	-	Average 3.83%; 1.94% positive in 2009 6.02% in 2012	N-MIT	**Utter *et al*., (2013), *Contact Dermatitis* 69, 231-238**
Human patch test, Finland, 2006-2008 Supportive study	10,821 dermatitis patients Dose: 1000 ppm or 0.1 % and 300 ppm or 0.03 % N-MIT	-	1.4 % positive at 1000 ppm (0.1 %) and 0.6 % at 300 ppm (0.03 %) N-MIT	N-MIT	**Ackermann *et al*., (2011), *Contact Dermatitis*, 64 (1), 49-53**
Human patch test, Sweden, May 2006- February 2010 Supportive study	2,536 dermatitis patients Dose: 2000 ppm or 0.2% N-MIT	-	1.5% on average were positive in 5 years (annual prevalence 1.1-2.2%) 30% of N-MIT-sensitized individuals were occupationally exposed to N-MIT, 45% (5/11) of them were painters	N-MIT	**Lundov *et al*., (2010) *Contact Dermatitis*, 63, 164-167**
Human patch study, 2009-2012 Leeds Center for Dermatology, UK Supportive study	Patients with contact dermatitis: -2009: 349; 0.02% N-MIT -2010:771; 0.02% N-MIT -2011:611; 0.02 % N-MIT and 238; 0.2% N-MIT -2012 (Jan-Jun):325; 0.02 and 0.2% N-MIT -2009: 0.6% (0.02% N-MIT) -2010: 1.1% (0.02% N-MIT) -2011: 1.8% (0.02% N-MIT), 3.8 % (0.2% N-MIT) -2012: 2.5% (0.02% N-MIT), 4.6% (0.2% N-MIT)			N-MIT	Urwin and Wilkinson, (2013), *Contact Dermatitis*, 68, 250-256
Analysis of human patch tests, Denmark	6,147 patients with contact dermatitis,	-	11/41 painters (27%) positive for N-MIT	N-MIT	Mose *et al*., (2012), *Contact Dermatitis*,

<table>
<tr><td>Supportive study</td><td>219 painters, 41 painters tested with N-MIT
Dose: not reported</td><td></td><td></td><td></td><td>67(5), 293-297</td></tr>
<tr><td>Repeated open application test (ROAT) and patch test were performed
Supportive study</td><td>11 patients sensitised to N-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% N-MIT, twice daily.

ROAT: 0.0007, 0.00035, 0.000035% N-MIT.

The use of cream protected with N-MIT was mimicked</td><td>-</td><td>Endpoint: Elicitation

Patch test:<table><tr><th>Dose (%)</th><th>Reaction</th></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><th>Dose (ppm)</th><th>Reaction</th></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>N-MIT</td><td>Lundov et al., (2011), Contact Dermatitis, 64, 330–336</td></tr>
</table>

A3.5.1 Short summary and overall relevance of the provided information on skin sensitisation

Read across:

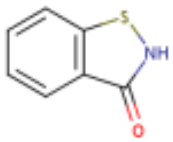
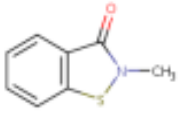
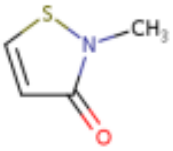
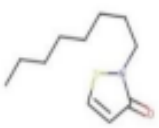
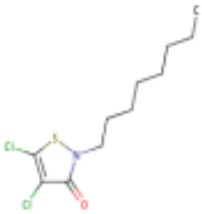
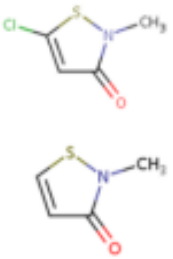
Read-across to data generated using N-MIT is proposed for this endpoint (skin sensitisation). N-MIT·HCl is water soluble and will dissociate in physiological conditions encountered in the skin to form N-MIT and HCl. The N-MIT component derived from N-MIT·HCl is chemically indistinguishable from N-MIT and will therefore have the same effects with regard to skin sensitisation. The adverse outcome pathway (AOP) for skin sensitisation is initiated by covalent binding to skin proteins, which is demonstrated for N-MIT, and will be unaffected by the presence of the HCl -component (which does not form covalent bonds with proteins). Subsequent AOP stages would also not be affected by the presence of HCl. When present in solid form (powder) or at high aqueous concentrations, N-MIT·HCl may cause more severe irritant/corrosive effects than N-MIT due to the HCl component; however, this will not influence sensitisation as the hydrogen and chloride ions are present physiologically and have no sensitisation potential. At low concentrations (i.e. those investigated experimentally) there

would be no effect of HCl. N-MIT is a potent skin sensitiser and has an agreed SCL for skin sensitisation of 0.0015%. No effect of the HCl component is predicted at this concentration level; consequently, the same SCL can reliably be used for N-MIT·HCl.

QSAR analysis using the OECD Toolbox (v. 4.1) was undertaken to provide a weight of evidence to for skin sensitisation potential of N-MIT·HCl. Visual inspection of the data in this category showed it to be relevant and robust. This was then used in the read-across approach taking the highest mode values from the 5 nearest neighbours with structural similarity to N-MIT to provide the estimate that N-MIT is predicted to be positive for skin sensitization potential *via* the likely mechanism of protein binding. The structures of the 5 nearest neighbours are all short to medium chain monoaldehydes with a single unsaturated bond and log Kow values bracketing that of N-MIT. Thus, it was concluded that the target chemical, N-MIT met the applicability domain used to provide a read-across estimation. In conclusion, the *in silico* estimates from the OECD Toolbox indicates that N-MIT is predicted to be a skin sensitizer based on protein binding, deemed to be an applicable domain. As protein binding provides the highest predictive power in correctly identifying potential skin sensitisers, no further work is considered necessary in addressing this endpoint.

For information also the data taken from RAC opinions on MBIT(2018), MIT (2016), OIT (2018), DCOIT(2018) and CIT/MIT (2016) on the skin sensitisation potential of MIT and other isothiazolinone compounds are presented in a Table A.22a.

Table A.24a: Comparation of skin sensitising properties of several isothiazolinones.

	BIT (CAS 2634-33-5)	MBIT (CAS 2527-66-4)	MIT (CAS 2682-20-4)	OIT (CAS 26530-20-1)	DCOIT (CAS: 64359-81-5)	CMIT/MIT (3:1) (CAS 55965-84-9)
Chemical structure						
LLNA	EC_3 = 29 % EC_3 = 32.4 % EC_3 = 2.3 % EC_3 = 4.8 % EC_3 = 10.4 %	EC_3 = 1.04 % EC_3 = 0.69 %	EC_3 = 0.86 %	EC_3 = 0.46 % EC_3 = 0.66 % EC_3 = 0.24 %	EC_3 = 0.03 %	EC_3 = 0.003 % EC_3 = 0.007 %
Potency	strong	strong	strong to extreme	strong to extreme	extreme	extreme
Classification	Skin Sens. 1A (this opinion)	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A
HRIPT	5/58 (9 %) at 725 ppm aq. (90.6 µg/cm²), 0/54 (0 %) at 360 ppm aq (45 µg/cm²) 5/45 (11 %) volunteers at 500 ppm (64.5 µg/cm²)* 0/111 at (500 ppm, (27.8 µg/cm²) in Rhoplex AC-64	9/45 (20 %) volunteers at 500 ppm*	1/116 (0.9 %) volunteers at 400 ppm (20 µg/cm²) 1/210 (0.5 %) at 500 ppm (25 µg/cm²)	0/103 subjects at 50 ppm (0.005 %) (2.5 µg/cm²) 1/222 (0.45 %) subjects at 100 ppm (0.01 %) (5 µg/cm²)	4/34 (12 %) at 250ppm (0.025 %) (12.5 µg/cm²) 14/34 (41 %) at 350ppm (0.035 %)	–
SCL	0.036 % (this opinion)	0.0015 %	0.0015 %	0.0015 %		0.0015 %

*From same study (Anonymous 1975)

A3.5.2 Comparison with the CLP criteria

N-MIT has been considered as a skin sensitizer according to RAC opinion adopted on March 2016 (CLH-O-0000001412-86-105/F). The proposal for classification Skin Sens. 1A, H317 has been agreed. A SCL ≥ 0.0015% (15 ppm) has been proposed and agreed for N-MIT.

In order to set a SCL for N-MIT, information was 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 animal studies reported in the CLH Opinion, were conducted to an appropriate regulatory standard, and a single study cannot be considered of better quality than any of the others. One GPMT showed N-MIT to have extreme potency whereas another GPMT was only indicative of this. In contrast, the available Buehler and LLNA tests showed N-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 N-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) could be justified. However, RAC agreed 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 was criticised for the use of water to dilute the test sample (50% MIT in propylene glycol); and 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) 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 considered that 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.

Interpretation of the remaining epidemiological data is less straightforward. RAC were concerned that there are an unusually high number of MIT sensitisation cases and that the frequency of such cases in the years preceding the Opinion had increased by up to 6-fold among consumers and workers that have been tested. RAC agreed with comments made during the public consultation that this appears to be linked to the introduction of MIT as a biocide, and specifically its use in cosmetics. Insufficient data were available to RAC for independent scrutiny, but it appeared 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 concluded that levels of MIT below 100 ppm have the potential to induce skin sensitisation. The SCCS had previously recommended that 15 ppm MIT would be a safe level in rinse-off cosmetics for protection of consumers from induction of skin sensitisation. RAC had not been provided with all the data underpinning this recommendation, but noted that comments received during the public consultation showed this view of the SCCS to be supported by various groups of expert dermatologists. Additionally, RAC noted that the SCCS (2015) concluded that there is "no adequate information to suggest a safe dose of MI [MIT] in leave-on cosmetic products from the view of induction of sensitisation, although circa 3.8 ppm, as present in MCI/MI [C(M)IT/MIT], may be indicative". In RAC's opinion, sufficient information was available to conclude that MIT has extreme potency. The results of the two guinea pig maximisation tests are consistent with the definition given in the CLP guidance. RAC agreed with the manufacturers of MIT who had 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 showed

that it was likely that levels below 100 ppm MIT will have the potential to induce sensitisation in humans.

RAC noted that this profile was 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 was be appropriate to set the same SCL for MIT as for C(M)IT:MIT (3:1), i.e. 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. 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 were considered to contradict the recommendation to set an SCL of 15 ppm; in both cases, the studies do not confirm the lowest level of MIT that could elicit responses. The DS also described four cases of allergic contact dermatitis to MIT evaporating from wall paints. It is not clear from the available information how these individuals concerned first developed 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 was 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 was therefore set as 1.5 ppm. RAC noted 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 was 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.

Based on read across, the same results can be applied for N-MIT·HCl. The criteria for classification as Skin Sens 1A, H317 are considered fulfiled.

A3.5.3 Conclusion on classification and labelling for skin sensitisation

Based on the read-across with N-MIT, a classification Skin Sens.1A (H317: May produce an allergic skin reaction) is proposed for N-MIT·HCl according to Regulation (EC) 1272/2008. In addition also a specific concentration limit ≥ 15 ppm is proposed.

A3.5.4 Overall conclusion on skin sensitisation related to risk assessment

Not applicable for the CLH report.

A.3.6. Respiratory sensitisation

No animal or human data on respiratory sensitisation are available.

A3.6.2 Comparison with the CLP criteria

No classification proposed for respiratory sensitisation due to lack of relevant data.

A3.6.3 Conclusion on classification and labelling for respiratory sensitisation

It can be concluded that in the absence of available studies in animals, specific investigations and reported cases of hypersensitivity in humans, N-MIT·HCl does not fulfil the CLP criteria for respiratory sensitisation

A3.6.4 Overall conclusion on respiratory sensitisation related to risk assessment

Not applicable for the CLH report.

A.3.7. Repeated dose toxicity/STOT RE

A.3.7.1. Short term repeated dose toxicity

A.3.7.2. Sub-chronic repeated dose toxicity

A3.7.2.1 Sub-chronic oral toxicity

Table A.25 Summary table of oral sub-chronic animal studies (usually 90-day studies)

Summary table of oral sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference

study						
OECD 409, 3 months, oral (diet), GLP, Reliability: 1 Key study	Dog, Beagle, ♂/♀, 4/sex/group	Kordek™ 573T (53.2% N-MIT) 0, 100/130, 400, 1500 ppm; daily diet	NOEL = 9.9 mg/kg bw/day for males and 11.1 mg/kg bw/d for females, (400 ppm) LOEL = 41 mg/kg bw/day equivalent to 1500 ppm	**1500 ppm**: lower terminal body weights in males (-9%) and females (-12%); lower overall food consumption in males (-8%) and females (-12%) due to lower intake (~50%) at the start of the study. Non-significantly lower RBC (-9%, -8%), Hb (-8%, -7%) and Hct (-9%, -7%) in males and females, respectively. Lower calcium in males (-6%, $p<0.05$) and females (-6%, NS). **400 ppm**: no effects **100/130 ppm**: no effects	N-MIT	Anonymous 20, 2004 (LoA)
OECD 408, 3 months, Oral (drinking water), GLP, Reliability: 1	Rat, Sprague-Dawley, ♂/♀, 10/sex/group	RH-573 Technical (97.8% N-MIT) 0, 75, 250, 1000 ppm; drinking water available *ad libitum*	NOEL = 19.0 mg MIT/kg bw/day in males and 24.6 mg/kg bw/day in females (250 ppm) LOEL = 66 mg/kg bw/day for males, 94 mg/kg bw/day for females (1000 ppm)	**1000 ppm:** Reduced body weight in males (-13%) and females (-10%); lower weight gain in males (-22%) and females (-23%); reduced food consumption in males (-20%); reduced water intake in males (-46%) and females (-45%). **250 ppm:** reduced food consumption (-14%) in males; reduced water consumption in males (-31%) and females (-43%), secondary to reduced food consumption **100 ppm:** reduced food	N-MIT	Anonymous 21, 2000 (LoA)

				consumption (-7%) and reduced water consumption in males (-21%); secondary to reduced food consumption		
OECD 407, 28 days, Oral (gavage)	Rat (Wistar), M/F, 5/sex/group	Acticide M50 (49% N-MIT) 0, 10, 28.6, 71.2 mg/kg bw/d N-MIT	LOAEL: 71.2 mg/kg bw/d NOAEL: 28.6 mg/kg bw/d	**71.2 mg/kg bw/d:** deaths (1M, 3F) Weeks 1-4; lethargy (Week 3-4), slightly reduced weight gain (Week 1-4); slightly reduced feed consumption (Week 1-4) **28.6 mg/kg bw/**d: no effects **10 mg/kg bw/**d: no effects	N-MIT	Anonymous 2, 2002 (in CLH Report for MIT, 2015)
90 days, Oral (gavage)	Rat (Wistar), M/F, 10/sex/group	Acticide M50 (50.5% N-MIT) 0, 7.52, 15.05, 30.09 mg/kg bw/d N-MIT	LOAEL: >30.09 mg/kg bw/d NOAEL: 30.09 mg/kg bw/d	Increased spleen weight was observed at 30.09 mg/kg bw/day. Other findings observed in this study were considered incidental and not adverse.	N-MIT	Anonymous 3, 2002 (in CLH Report for MIT, 2015)

No human data are available.

A3.7.2.2 Sub-chronic dermal toxicity

Table A.26 Summary table of dermal sub-chronic animal studies (usually 90-day studies)

Summary table of dermal sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study,	Species, Strain, Sex,	Test substance (including purity), Vehicle,	NOAEL, LOAEL	Results (all dose levels including severity and	Remarks (e.g. major deviations)	Reference

Guideline, GLP status, Reliability, Key/supportive study	No/ group	Dose levels, Surface area, Duration of exposure		magnitude of all effects, including target organs)		
Similar to OECD 411, 3 months, dermal, GLP, Reliability: 1 Key study	Rabbit, New Zealand White, ♂/♀, 6/sex/group	Kathon™ 886 MW (14.6 % CMIT/MIT, equiv.3.65% N-MIT and 10.95% CMIT) 0, 100, 200, 400 ppm; 5 d/week	NOAEL = not determined LOAEL = 100 ppm a.i. (0.1 mg a.s./kg bw/day)	400 ppm: deaths* (3M, 3F); erythema mean score (Weeks 2/5/7/13) 0.7/0.8/1.5/3.0; oedema 0/0/0/0.5 200 ppm: deaths* (1M, 4F); erythema mean score (Weeks 2/5/7/13) 0.07/0.0/1.0/1.5; oedema 0/0/0/0.1 100 ppm: deaths* (0M, 3F); erythema mean score (Weeks 2/5/7/13) 0.0/0.0/0.09/1.9; oedema 0/0/0/0.1 *25-40% of rabbits died after 6-8 weeks of treatment, in the low, mid- and high-dose groups, due to pleuritis and alveolar edema. However, it cannot be ruled out that this effect	Study performed with CMIT/MIT There were 6 animals/sex/group rather than the suggested 10/sex/group.	Anonymous 24, 1982 (LoA)

				is treatment related. No control rabbits died from lung disease.		

No human data are available.

A3.7.2.3 Sub-chronic inhalation toxicity

Table A.20 Summary table of inhalatory sub-chronic animal studies (usually 90-day studies)*

Summary table of inhalatory sub-chronic animal studies (usually 90-day studies)						
Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body/ head only), Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
OECD 413, 3 months, Inhalation, GLP, Reliability: 1 Key study	Rat, Sprague Dawley, ♂/♀, 16/sex/group	Kathon™ 886 MMP Process (14.6 % CMIT/MIT) 0, 0.34, 1.15, 2.64 mg/m^3 6 h/d, 5d/week, nose only	NOAEC = 0.34 mg CMIT/MIT/m^3 (0.00034 mg/L) based on irritation to the respiratory tract; LOAEC = 1.15 mg/m^3 (0.00115	**2.64 mg/m^3**: chromorhinorrhea, rhinorrhea, eye squint, bradypnea, dyspnea; reduced body weight gain; reduced serum protein (F); reduced spleen weight (M); slight to moderate incidences of	CMIT/MIT In the original report only a summary of clinical signs is stated, but no raw data are	Anonymous 22, 1984 (LoA)

			mg/L)	eosinophilic droplets in the anterior respiratory mucosa of the nasal turbinate, slight rhinitis in the lining of the anterior portion of the nasal cavity (M, F) **1.15 mg/m³: none** **0.34 mg/m³: none**	presented in this study.	

No human data are available.

A3.7.2.4 Overall conclusion on sub-chronic repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.3. Long-term repeated dose toxicity

A3.7.3.1 Long-term oral/dermal/inhalation toxicity

Table A.28 Summary table of chronic toxicity studies in animals

Summary table of chronic toxicity studies in animals						
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
OECD 453, 24 months, Oral	Rat, Sprague	Kathon™ 886 (14.2%	LOAEL systemic was not determined.	**300 ppm:** lower body weight; reduced food	Study performed	Anonymous 29, 1994

(drinking water), GLP, Reliability: 1	Dawley, ♂/♀, 80-90/sex	CMIT/MIT), 0, 30, 100, 300 ppm	NOAEL systemic = 17.2 mg/kg bw/day in males and 25.7 mg/kg bw/day in females (300 ppm) NOAEL local effects = 2.0 mg/kg bw/day in males and 3.1 mg/kg bw/day in females (30 ppm)	consumption; reduced water consumption (15-40%); gross gastric irritation (forestomach thickened, hyperkeratosis) (M, F); gastric glandular mucosa histopathology (focal necrosis, oedema, inflammatory cell infiltration), hyperplasia (18/30 M, 8/19 F) **100 ppm:** reduced water consumption (3-30%); gross gastric irritation (forestomach thickened, hyperkeratosis) (M, F); gastric glandular mucosa histopathology (focal necrosis, oedema, inflammatory cell infiltration), hyperplasia (3/25 M, 5/21 F) **30 ppm:** reduced water consumption (0-22%)	with CMIT/MIT	(LoA)
OECD 452, 30 months, dermal, GLP, Reliability: 1	Mouse, CD-1, M, 40/gp	Kathon™ CG (1.5% CMIT/MIT) 0, 400 ppm; 3/week	Systemic NOAEL was not determined. Dermal NO(A)EL for tumour induction >400 ppm (10 µg/animal).	**400 ppm:** brown staining of application site (9/40), eschar/ desiccation/flaking of skin (10/40); dermal histopathology: focal or multifocal epidermal	Study performed with CMIT/MIT	Anonymous 30, 1983 (LoA)

				necrosis (12/40), hyperplasia (28/40), hyperkeratosis (7/40), eschar (5/40), dermal inflammation (6/40), increased dermal collagen (30/40)		

A.3.7.4. Specific target organ toxicity – repeated exposure (STOT RE)

A3.7.4.1 Short summary and overall relevance of the provided information on STOT RE

Oral studies

The sub-chronic toxicity of N-MIT by the oral route was investigated in a 90-day study in rats (Data summarized in Table A.28; Anonymous 21, 2000) Rats received 0, 75, 250 and 1000 ppm N-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 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, oesophagus, 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. A NOAEL of 19 and 25 mg/kg bw/d (250 ppm) for males and females, respectively, was determined in this study based on decreased body weight and food consumption at 1000 ppm. No systemic toxicity was observed.

In a sub-chronic oral toxicity study in the dog (Data summary in Table A.25; Anonymous 20, 2004), N-MIT was administered daily in the diet for 90 days at 100/130, 400 and 1500 ppm. 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. A NOAEL of 9.9 and 11 mg/kg bw/d N-MIT (400 ppm) was determined for males and females, respectively, based on decreased food consumption and body weight. The LOAEL was 41 mg/kg bw/d (1500 ppm). No systemic toxicity was observed in this study.

Acticide M50 (49% N-MIT in water) was administered to rats by gavage for 28 consecutive days at 0, 10, 28.6 and 71.2 mg N-MIT/kg

bw/day according to the OECD Guidance 407 (Data summary in Table A.25; Anonymous 2, 2002, in CLH Report for MIT, 2015). 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 this 28-day oral toxicity study in rats a LOAEL of 71.2 mg/kg bw/d N-MIT and a NOAEL of 28.6 mg/kg bw/d were determined.

In an oral subchronic toxicity study in rats, the animals were administered Acticide 50 M, containing 50.7% N-MIT in water, by gavage at 7.52, 15 and 30 mg/kg bw/d N-MIT for 90 consecutive days (Data summary in Table A.25; Anonymous 3, 2002, in CLH Report for MIT, 2015). 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 N-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 N-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 N-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 N-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 hyperplasia in both sexes. The effects observed were not considered to be treatment-related. From this study a NOAEL of 30.1 mg/kg bw/d N-MIT was derived.

In a combined chronic toxicity/carcinogenicity study in the rat, groups of 80-90 rats/sex/group were administered CMIT/MIT (14.2%) in the drinking water at concentrations of 0, 30, 100 or 300 ppm for up to 24 months (Data summary in Table A.28; Anonymous 29, 1994). Reduced food consumption was seen in male rats exposed to 300 ppm. A treatment-related and concentration-dependent decrease in water consumption was seen in both sexes in all treated groups throughout the study. These decreases ranged from 0-22% at 30 ppm, 3-30% at 100 ppm and 15-40% at 300 ppm.
The decreases appear to be due to the unpalatability of the test material. Haematology, clinical chemistry and urinalysis did not reveal any effects of treatment. There were no effects on organ weight. Treatment-related morphologic changes were limited to the stomach and occurred in both sexes at 100 and 300 ppm. The primary effect noted was gastric irritation which was reflected by thickening of the forestomach mucosa due to hyperplasia and hyperkeratosis of the squamous mucosa. Focal necrosis of the superficial glandular mucosa and edema and inflammatory cell infiltration in the forestomach submucosa were seen in the 300 ppm males. No adverse effects were observed on the gross and histopathology of any tissues/organs distant from the site of dosing at any dose level. A NOAEL of 30 ppm (2.0/3.1 mg/kg bw/d) can be determined for this study.

Dermal studies

Groups of rabbits (6/sex/group) were dermally exposed CMIT/MIT (~14%) on 5 days/week in a 90-day study at concentrations of 0, 100, 200 and 400 ppm (Data summarized in Table A.26; Anonymous 24, 1982). A number of deaths occurred in this study across all treated groups, but not in controls. Deaths are attributed to pulmonary disease. A concentration-related increase in local dermal effects (slight to severe erythema, slight oedema) was seen in all treated groups. There was no evidence of systemic toxicity in this study. Necropsy revealed minimal to slight sub-epidermal cell infiltrate in 8/12 rabbits at 400 ppm. A NOAEL was not determined for this study due to evidence of local effects at all tested concentrations; however, there was no evidence of systemic toxicity in this study.

A 30-month dermal carcinogenesis study was performed with CMIT/MIT (~14%) in the mouse (Data summary in Table A.28; Anonymous 30, 1983). Groups of 40 male mice were exposed to the test material at a single concentration of 400 ppm, on three days per week. Survival was unaffected by treatment. Effects of treatment in this study were limited to brown staining, eschar, flaking and/or desiccation of the application site. Histopathology of the application site skin revealed focal or multifocal epidermal necrosis, hyperplasia,

hyperkeratosis, eschar, dermal inflammation, and increased dermal collagen.

Inhalation study

In a 90-day repeated exposure inhalation study, groups of rats were exposed to atmospheres containing CMIT/MIT (~14%) for hours/day on five days/week at concentrations of 0, 0.34, 1.15 and 2.64 mg/m^3 (Data summarized in Table A.27; Anonymous 22, 1984). Rats exposed to 2.64 mg a.i./m^3 exhibited signs resulting from exposure consistent with those produced by a sensory irritant (chromorhinorrhea, rhinorrhea, eye squint, bradypnea, dyspnea). Decreased body weight gains were apparent in rats exposed to 2.64 mg/m^3. There were no effects on haematological parameters; clinical chemistry revealed decreased serum protein in females at 2.64 mg/m^3. Decreased male spleen weights were seen at 2.64 mg/m^3. Gross pathology did not reveal any effects of exposure. Histopathological evaluation showed slight to moderate incidences of eosinophilic droplets in the anterior respiratory mucosa of the nasal turbinates and slight rhinitis in the lining of the anterior portion of the nasal cavity were observed in the 2.64 mg/m^3 treated animals. All the histopathologic changes were very minor, potentially reversible, and generally reflective of minimal tissue responses to a very mild, low-grade respiratory irritant. No adverse effects were seen on the histopathology of any tissues/organs distant from the site of dosing. A LOAEC of 1.15 mg/m^3 was determined for this study based on slight, treatment-related rhinitis; The NOAEC was 0.34 mg/m^3. There were no systemic effects in this study. Rats at the highest exposure level of 2.64 mgm^3 exhibited very mild, low grade respiratory irritation. There were no adverse effects on the histopathology of any tissues or organs distant from the site of exposure.

By read-across with N-MIT and CMIT/MIT, no significant primary systemic toxic effects occurred at any dose. The effects observed were either restricted to reduced body weights (oral) or local effects (rhinitis in the inhalation and skin irritation in the dermal studies) with no functional change observed. The effects observed on body weight gain and food consumption are considered as secondary to the local toxicity. Consequently, STOT RE is not relevant.

A3.7.4.2 Comparison with the CLP criteria

As described above, after repeated exposure no significant systemic toxic effects occurred at any dose. The effects observed were either restricted to reduced body weights (oral) or local effects (rhinitis in the inhalation and skin irritation in the dermal studies) with no functional change observed. The effects observed on body weight gain and food consumption are considered as secondary to the local toxicity. Considering there is no evidence of primary systemic toxicity, STOT RE is not relevant.

A3.7.4.3 Conclusion on classification and labelling for STOT RE

According to Regulation (EC) 1272/2008 the classification of N-MIT·HCl with STOT-RE is not warranted.

A.3.8. Genotoxicity / Germ cell mutagenicity

A.3.8.1. In vitro

Table A.29 Summary table of *in vitro* genotoxicity studies

Summary table of in vitro genotoxicity studies					
Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains)	Results (including cytotoxicity and +/- S9 mix)	Remarks (e.g. major deviations)	Reference
Bacterial reverse gene mutation assay, OECD 471	Kordek 573T (97.5% N-MIT) 5-1000 µg/plate	*Salmonella typhimurium*, TA 1535, TA 1537, TA 98, TA 100, TA 102	Not mutagenic. Toxicity was observed in the definitive assay in all strains at 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.	N-MIT	Anonymous 32, 1999 (in CLH Report for MIT 2015)
Bacterial reverse gene mutation assay, OECD 471	Acticide SR3267 (49% N-MIT): 8-648 µg/plate (3.9-317.5 µg/plate N-MIT)	*Salmonella typhimurium*, TA 1535, TA 1537, TA 98, TA 100	Not mutagenic. Toxicity was observed at 648 µg/plate in the plate incorporation assay; and at 72 µg/plate in the pre-incubation	N-MIT	Anonymous 4, 2000 (in CLH Report for MIT, 2015)

			assay (+/-S9)		
Bacterial reverse gene mutation, OECD 471 (1997), GLP, Reliability: 1, Key study	N-MIT·HCl (98.7% purity), DMSO, 0-5000 µg/plate	Five *S.typhimurium* strains used (TA98, TA100, TA1535, TA1537, TA102) +/-S9 Plate incorporation and pre-incubation methodology	N-MIT·HCl did not induce mutation in five histidine-requiring strains +/-S9, up to the limit of toxicity	N-MIT·HCl	Anonymous 7, 2018 (unpublished report no.: 8366777)
Cytogenetic study in CHO cells, OECD 473, GLP, Reliability: 1	Kordek™ 573T (97.5% N-MIT), DMSO, 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 with S9.	Chinese hamster ovary (CHO) cells	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.	N-MIT	Anonymous 8, 2000 (LoA)
Cytogenetic study in CHO cells, OECD 473	Acticide M50 (49.5% N-MIT); 2.5, 5, 10 µg/mL(1.3, 2.5 and 5 µg/ml N-MIT)	Cultured primary human lymphocytes	Not mutagenic. N-MIT did not induce chromosomal aberrations and mitotic index in the absence or presence of S9 mix (5 and 15%)	N-MIT	Anonymous 33, 2002 (in CLH Report for MIT, 2015)
Gene mutation study (HGPRT) in CHO cells, OECD 476, GLP, Reliability: 1	Kordek™ 573T (97.5% N-MIT), DMSO, Definitive assay: 0.5, 1.0, 5.0, 10, 15 and 25 µg/ml; confirmatory	Chinese hamster ovary CHO cells (examining hprt locus)	Not mutagenic in Chinese hamster ovary cells +/-S9	N-MIT	Anonymous 9, 2000 (LoA)

	assay: 5.0, 10, 15, 25 and 40 µg/ml				
Gene mutation study (HPRT) in mammalian cells, OECD 476	Acticide SR 3267 (49% N-MIT) in water) First test 0.25-4.0 µg/ml (+/-S9); second test: 1.0-5.0 µg/ml +/- S9	Chinese hamster ovary CHO cells (examining hprt locus)	Not mutagenic. No increase in mutant frequencies was observed in the presence or absence of metabolic activation	N-MIT	Anonymous 34, 2000 (in CLH Report for MIT, 2015)

A.3.8.2. In vivo

No *in vivo* data are available for N-MIT·HCl; *in vivo* data were not submitted in the biocidal active substance dossier as they were not required in the absence of any evidence of genotoxicity i*n vitro*. Nevertheless, in vivo data are available for N-MIT and are summarised below (taken from the CLH Report).

Table A.30 Summary table of *in vivo* genotoxicity studies

Summary table of in vivo genotoxicity studies					
Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Results	Remarks	Reference
Micronucleus in bone marrow erythrocytes, OECD 474	N-MIT (97.5% purity) 0, 10, 50, 100 mg/kg bw	Mouse CD-1, 5/sex/group (7/sex/group in high dose) Single oral dose (gavage); sampling 24 and 48 h after treatment	Not mutagenic	At the highest dose two females showed clinical signs and were found dead 24 hours after exposure.	Anonymous 35, 2000 (in CLH Report for MIT, 2015)
Micronucleus in bone marrow	Acticide SR containing N-MIT	Mouse CRL:NM RI BR, 5/sex/group	Not mutagenic	Formation of micronuclei	Anonymous 36, 2000 (in CLH

erythrocytes, OECD 474	(49.8%) 0, 100, 150, 200 mg/kg bw Acticide SR 3267; corresponding to 0, 49.8, 74.4, 99.6 mg/kg bw N-MIT	Single oral dose (gavage); sampling 24 and 48 h after treatment		was not induced after exposure to MIT	Report for MIT, 2015)
Unscheduled DNA Synthesis, OECD 486	N-MIT (51.1%) 0, 103, 206, 308 mg/kg bw (N-MIT)	Rat, Crl:CD(SD)IGS, males and females for range-finding assay, males for definitive assay¸4 males/dose; 6 males for high dose	Not mutagenic	Salivation and hypoactivity were observed in treated rats	Anonymous 37, 2003 (in CLH Report for MIT, 2015)
Unscheduled DNA Synthesis, OECD 486	Acticide 14 (CIT/MIT (3:1): 13.9 % w/w, aqueous solution CIT, 5- chloro-2-methyl-4-isothiazolin3-one, CAS 26172-55-4: 10.2 % and MIT, 2- methyl-4-isothiazolin3-one; CAS 2682-20-4: 4.0 %)	Rat Wistar, male, 6/dose Single oral dose (gavage) of 14 % CMIT/MIT mixture (3:1); sampling 2-4 h and 12-14h after treatment 0, 19, 60 mg/kg bw Acticide 14, corresponding to 0, 2.64, 8.34 mg/kg bw CMIT/MIT (3:1)	Not mutagenic	No increase in UDS was observed	Anonymous 38, 1994 (in CLH Report for MIT, 2015)

No human data are available.

A3.8.2.1 Short summary and overall relevance of the provided information on germ cell mutagenicity.

Bacterial reverse gene mutation

A study on mutagenicity in bacteria was conducted on *Salmonella typhimurium* strains TA98, TA100, TA1535; TA1537 and TA102 with and without metabolic activation (S9 fraction). At the highest concentrations used cytotoxicity was observed in bacteria exposed to N-MIT in the presence or absence of S9. N-MIT did not increase the number of revertant colonies under conditions of this test (Table 29, Anonymous

32, 1999, in CLH Report for MIT, 2015). 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. An 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 (Table 29, Anonymous 4, 2000, in CLH Report for MIT, 2015). *S. typhimurium* strain TA102 or *Escherichia coli* WP2 were not used in the test as it should have been according to 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 N-MIT/plate, in a plate incorporation assay and pre-incubation assay (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 the pre-incubation assay were visible as decreasing revertant numbers at concentrations of 216 µg/plate (105.84 µg N-MIT/plate), and as impaired background growth at concentrations of >72 µg/plate (35.28 µg N-MIT/plate) without S9-mix. In the direct plate incorporation method growth inhibition effects were visible as decreasing revertant numbers and impaired background growth at concentrations of 648 µg/plate (317.5 µg N-MIT/plate) without S9-mix. Under the test conditions N-MIT is not mutagenic in *Salmonella typhimurium* strains TA98, TA 100, TA 1535 and TA 1537 with and without metabolic activation.
N-MIT·HCl did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations limited by toxicity in the absence and in the presence of a rat liver metabolic activation system (S9), utilizing the plate incorporation methodology (absence and presence of S9) or pre-incubation methodology (presence of S9) (Table A.29, Anonymous 7, 2018).

Mammalian structural and numerical chromosome changes

N-MIT was also tested in Chinese hamster ovary cells for induction of chromosomal aberrations. An increase in the number of chromosomal aberrations was observed at concentrations of N-MIT that exerted cytotoxic activity what was indicated by reduction of mitotic index or cell count (27-56%). The result is considered to be a false positive (Table A.29, Anonymous 8, 2000, in CLH Report for MIT, 2015). Acticide M 50 (2.5, 5 and 10 µg/ml, corresponding to 1.3, 2.5 and 5 µg N-MIT/ml) was tested in an *in vitro* mammalian chromosomal aberration test in human lymphocyte culture (Table A.29, Anonymous 33, in CLH Report for MIT, 2015). Cytotoxicity data of preliminary study showed that 10 µg/ml could be the highest dose used in the main test. Lymphocytes were exposed to N-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 long-term exposure periods. 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.

N-MIT was negative in an *in vitro* gene mutation study (HGPRT) in Chinese hamster ovary cells with and without metabolic activation (Table A.29, Anonymous 9, 2000). Concentrations were tested up to the cytotoxic level. The data obtained confirmed N-MIT did not cause a significant increase in the mutant frequency at the HGPT locus among the test-article treated cultures in the presence and absence of exogenous metabolic activation. There was no dose-dependent response in the test article treated cultures. The mutant frequencies of the test article solvent and the solvent for the positive controls were within laboratory's historical negative control values. The positive controls

caused a significant increase in the mutant frequencies, thus demonstrating the sensitivity and specificity of the assay. All criteria for a valid assay were met. Thus, N-MIT was considered to be non-mutagenic in CHO cells when tested under the conditions of this study. The frequency of HGPRT mutants was also evaluated in CHO cells after treatment with 0.25, 0.5, 1, 2 and 4 µg/mL Acticide SR 3267 in the first test (corresponding to 0.124, 0.249, 0.498, 0.996 and 1.992 µg/mL N-MIT); 1, 2, 4 and 5 µg/mL Acticide SR 3267 (corresponding to 0.498, 0.996, 1.992 and 2.49 µg/mL N-MIT) in the second test (Table A.29, , Anonymous 34, 2000, in CLH Report for MIT, 2015). Both tests were performed in the presence and absence of S9 mix. Cytotoxicity was observed at 5.00 µg/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.

In vivo data

No *in vivo* data are available for N-MIT·HCl; *in vivo* data were not submitted in the biocidal active substance dossier as they were not required in the absence of any evidence of genotoxicity i*n vitro*. Nevertheless, *in vivo* data are available for N-MIT and are summarised above (Table A.30, taken from the CLH Report). No evidence of micronucleus formation was seen in male and female CD-1 mouse bone marrow erythrocytes in a study performed with N-MIT at dose levels of up to 100 mg/kg bw (Table A.30, Anonymous 35, 2000, in CLH Report for MIT, 2015). No evidence of micronucleus formation was seen in male and female NMRI mouse bone marrow erythrocytes in a study performed with Acticide SR (containing 49.8% N-MIT) at dose levels of up to 99.6 mg/kg bw N-MIT (Table A.30, Anonymous 36, 2000, in CLH Report for MIT, 2015). No evidence of unscheduled DNA synthesis (UDS) was seen in male and female SD rat liver in a study performed with a formulation containing 51.1% N-MIT at dose levels of up to 308 mg/kg bw N-MIT (Table A.30, Anonymous 37, 2003, in CLH Report for MIT, 2015). No evidence of UDS was seen in male Wistar rat liver in a study performed using Acticide 14 (containing 13.9% CMIT/N-MIT (3:1)) at dose levels of up to 60 mg/kg bw Acticide 1e (8.34 mg/kg bw CMIT/N-MIT) (Table A.30, Anonymous 38, 1994, in CLH Report for MIT 2015).

Read across:

Read-across to data generated using N-MIT is proposed for two studies addressing this endpoint (genotoxicity). The read-across is justified on the basis that the target substance (N-MIT·HCl) will dissociate to form N-MIT (the source substance) and HCl in the aqueous environment used in all studies *in vitro,* and in the stomach of animals dosed orally. The source and target substances are therefore identical. The HCl generated from the dissociation of N-MIT·HCl is indistinguishable from the HCl present in the culture media or stomach. HCl further dissociates in aqueous conditions to the hydrogen and chloride ions, both of which are physiologically ubiquitous and subject to homeostatic control. There is therefore no concern relating to the genotoxicity of the HCl component. The presence of additional HCl in the *in vitro* and *in vivo* test systems resulting from the dissociation of N-MIT·HCl in the culture medium or stomach may have the effect of limiting the concentrations able to be tested due to effects on pH. The pH of the culture medium in studies using mammalian cells is used as a limiting factor in terms of the highest concentrations tested. Consequently, the used of data generated using N-MIT is likely to be a worst case as the studies in mammalian cells are likely to have been performed with higher concentrations than would have been achievable with N-MIT·HCl. The use of data generated using N-MIT is adequate for the genotoxicity endpoint.

A3.8.2.2 Comparison with the CLP criteria

There was no indication that N-MIT / N-MIT·HCl has a mutagenic effect on somatic cells in the Ames and mammalian gene mutation *in vitro* assays. An increase in the incidence of chromosome aberrations in Chinese hamster ovary cells was observed. The increase in chromosomal aberrations was only observed at concentrations that induced significant cytotoxicity (expressed either as a reduction in cell count or as reduction in mitotic index). A highly non-linear dose response for the induction of chromosomal aberrations and the associated correlation of the chromosomal aberrations with high level of cytotoxicity suggest that the chromosomal damage was induced by an indirect process associated with cytotoxicity. There is no evidence of bone marrow erythrocyte micronucleus formation in studies in the mouse *in vivo*, and no evidence for the induction of unscheduled DNA synthesis in the liver in studies in the rat *in vivo.*

The criteria for classification for mutagenicity were not met, therefore no mutagenicity classification is proposed.

A3.8.2.3 Conclusion on classification and labelling for germ cell mutagenicity

No *in vivo* studies in germ cells have been conducted on N-MIT·HCl / N-MIT because the test article is not genotoxic in somatic cells *in vitro* or *in vivo*. By read-across and considering negative results *in vitro* and *in vivo* studies, classification criteria according to Regulation (EC) 1272/2008 are not met, therefore no classification is required.

A3.8.2.4 Overall conclusion on genotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.9. Carcinogenicity

Table A.31 Summary table of carcinogenicity studies in animals

Summary table of carcinogenicity studies in animals						
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability,	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels, Duration of	NOAEL, LOAEL	Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels)	Remarks (e.g. major deviations)	Reference

Key/supportive study		exposure				
OECD 453, 24 months, Oral (drinking water), GLP, Reliability: 1	Rat, Sprague Dawley, ♂/♀, 80-90/sex	Kathon™ 886 (14.2% CMIT/MIT), 0, 30, 100, 300 ppm	LOAEL systemic was not determined. NOAEL systemic = 17.2 mg/kg bw/day in males and 25.7 mg/kg bw/day in females (300 ppm) NOAEL local effects = 2.0 mg/kg bw/day in males and 3.1 mg/kg bw/day in females (30 ppm)	**300 ppm:** lower body weight; reduced food consumption; reduced water consumption (15-40%); gross gastric irritation (forestomach thickened, hyperkeratosis) (M, F); gastric glandular mucosa histopathology (focal necrosis, oedema, inflammatory cell infiltration), hyperplasia (18/30 M, 8/19 F) **100 ppm:** reduced water consumption (3-30%); gross gastric irritation (forestomach thickened, hyperkeratosis) (M, F); gastric glandular mucosa histopathology (focal necrosis, oedema, inflammatory cell infiltration), hyperplasia (3/25 M, 5/21 F) **30 ppm:** reduced water consumption	CMIT/MIT	Anonymous 29, 1994 (LoA)

				(0-22%) No evidence of carcinogenicity; no evidence of histopathology beyond the site of contact (stomach)		
OECD 452, 30 months, dermal, GLP, Reliability: 1	Mouse, CD-1, M, 40/gp	Kathon™ CG (1.5% CMIT/MIT) 0, 400 ppm; 3/week	Systemic NOAEL was not determined. Dermal NO(A)EL for tumour induction >400 ppm (10 µg/animal).	**400 ppm:** brown staining of application site (9/40), eschar/ desiccation/flaking of skin (10/40); dermal histopathology: focal or multifocal epidermal necrosis (12/40), hyperplasia (28/40), hyperkeratosis (7/40), eschar (5/40), dermal inflammation (6/40), increased dermal collagen (30/40) No evidence of carcinogenicity; no evidence of histopathology beyond the site of contact (skin)	CMIT/MIT	Anonymous 30, 1983 (LoA)

No human data are available.

No other data are available.

A3.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Carcinogenicity data has been obtained through a letter of access to address this endpoint.

Combined chronic toxicity/carcinogenicity study:

Groups of 90 males and 80 female rats were orally administered Kathon™ 886 (14.2% CMIT/MIT) in drinking water at doses of 30, 100, 300 ppm for 24 months (Table A.31, Anonymous 29, 1994 (LoA)). Clinical observations, bodyweights, food consumption, water consumption, haematology and clinical biochemistry (blood and urine), were measured at the interim and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem.* Terminal blood samples were taken, selected organs were weighed and specified tissues were taken for subsequent histopathology examination. Decreases in drinking water consumption, although not considered an adverse toxicological effect, were observed at all concentrations of chloro-methylisothiazolone/methylisothiazolone tested (30, 100 and 300 ppm CMIT/MIT in drinking water). Decreases in body weight and feed consumption were observed at the highest dose tested (300 ppm CMIT/MIT or 17 to 26 mg CMIT/MIT/kg body weight/day) and were considered secondary to decreases in water consumption. Gross and microscopic changes produced by chloro-methylisothiazolone /methylisothiazolone were limited to the stomach (gastric irritation at site of dosing) in the mid-dose and high-dose groups. No evidence of systemic toxicity (i.e., no adverse effects on the histopathology of any tissues/organs distant from the site of dosing) was observed at dose levels up to and including the high dose (300 ppm CMIT/MIT or 17 to 26 mg CMIT/MIT/kg body weight/day). Chloro-methylisothiazolone/methylisothiazolone produced no evidence of carcinogenicity at doses up to and including the highest dose tested (300 ppm a.i or 17 to 26 mg CMIT/MIT/kg body weight /day). NOAEL 2.0 mg and 3.1 mg C(M)IT/MIT/kg bw/day for local effects was derived in males and in females, respectively, and NOAEL for systemic effects was 17.2 mg/kg bw/day in males and 25.7 mg/kg bw/day in females.

Chronic dermal toxicity study:

The test article Kathon™ CG (1.5% CMIT/MIT) was applied dermally to the closely clipped skin on the backs of male CD-1 mice (40 animals/group) at a concentration of 400 ppm CMIT/MIT and at a dose of 25 µL 3 times per week for 30 months (Table A.31, Anonymous 30, 1983 (LoA)). Clinical signs of_toxicity, mortality, effects on body weight and dermal findings were evaluated. No local or tumorigenic potential was observed at 400 ppm. No adverse effects were seen on the histopathology of any tissues/organs distant from the site of dosing. NOAEL for tumour induction is >400 ppm (10 mg/animal). Overall NOAEL was not determined in this study.

Read-across:

Read-across to data generated using N-MIT is proposed for this endpoint (carcinogenicity). The read-across is justified on the basis that the target substance (N-MIT·HCl) will dissociate to form N-MIT (the source substance) and HCl in the aqueous environment of the stomach following oral dosing. The source and target substances are therefore identical. The HCl generated from the dissociation of N-MIT·HCl is indistinguishable from the HCl produced by the stomach in significant quantities. HCl further dissociates in aqueous conditions to the hydrogen and chloride ions, both of which are physiologically ubiquitous and subject to homeostatic control. The systemic toxicity of N-MIT·HCl and N-MIT will therefore be identical. While it is theoretically possible that the local effects of N-MIT·HCl on the stomach may be more marked as a consequence of dissociation of the molecule to form HCl, this would appear to be unlikely based on the relatively low dose levels used in the studies and the normal presence of quantities of HCl in the stomach. In any case, a more severe local effect produced by N-MIT·HCl would further limit the dose levels used in the studies, resulting in a lower level of systemic exposure to N-MIT. A local

carcinogenic effect following the chronic administration of a corrosive substance would also not be considered to be of relevance to classification or to the human risk assessment. In this respect, the use of data generated using N-MIT is adequate for the carcinogenicity endpoint. C-MIT is structurally similar to N-MIT and has a comparable toxicological profile, driven by local effects; read-across to the CMIT/MIT mixture is additionally justified on this basis.

A.3.9.2 Comparison with the CLP criteria

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 1A is reserved for substances known to have carcinogenic potential in humans. Since there is no evidence of N-MIT·HCl having caused carcinogenicity in humans, classification in Category 1A is not justified.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 1B is reserved for substances that are presumed to be carcinogenic in humans and is largely based on data from animal studies where there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 2 is reserved for substances where there is evidence obtained from human and/or animal studies but which is not sufficiently convincing to place the substance in Category 1.

No evidence of carcinogenicity has been observed in animal studies after oral and dermal administration. The classification criteria are not met.

A.3.9.3 Conclusion on classification and labelling for carcinogenicity

As there is insufficient evidence for a carcinogenic effect in rats and mice, and there are no other concerns about the potential carcinogenicity of N-MIT·HCl, no classification is proposed according to Regulation (EC) 1272/2008.

A.3.9.4 Overall conclusion on carcinogenicity related to risk assessment

Not applicable for the CLH report.

A.3.10. Reproductive toxicity

A.3.10.1. Sexual function and fertility

Table A.32 Summary table of animal studies on adverse effects on sexual function and fertility

Summary table of animal studies on adverse effects on sexual function and fertility						
Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
3-gen, OECD 416, Oral (drinking water), GLP, Reliability: 1	Rat, Sprague-Dawley, ♂/♀, 30/sex/group	Kordek™ 573F (51.4% N-MIT) 0, 50, 200, 1000 ppm 70 days prior to pairing, through mating, gestation, lactation of 2 litters	**NO(A)EL: Parental: 200 ppm (♂/♀: 15/22 mg/kg bw/d)** Reproductive: 1000 ppm (♂/♀: 69/93 mg/kg bw/d) Offspring: 200 ppm (♂/♀: 15/22 mg/kg bw/d)	**1000 ppm:** Reduced body weight gain during pre-mating (F0; –10%,-6%), (F1; -1%, -12%) (M, F); during gestation (-14%, -14%) (F0, F1 females); increased weight gain (+37%) during lactation (F0 females). Reduced food consumption during gestation (-9%), -9%) and lactation (-11%, -12%) (F0, F1 females). Reduced water consumption during pre-mating in F0 (-32%, -32%) and F1 (-32%, -31%) (M, F); reduced water consumption during gestation (-41%, -41%) and lactation (-25%, -27%) (F0, F1 females). Lower F1 pup weight at birth (-6%), reduced pup weight gain during lactation (F0, F1).	N-MIT	Anonymous 27, 2003 & Anonymous 28, 2007 (LoA)

				Delayed time of F1 sexual maturation (M; PPS 46.9 compared to 44.4 days) and F (VO 35.6 compared to 33.0 days); secondary to body weight effects (no effects on weight at attainment of PPS/VO). No effects on AGD (F2) **200 ppm**: Reduced water consumption during pre-mating in F0 (-18%, -16%) and F1 (-9%, -11%) (M, F); reduced water consumption during gestation (-21%, -19%) and lactation (-11%, -9%) (F0, F1 females). **50 ppm:** Reduced water consumption during pre-mating in F0 M (-9%). No effect on fertility or reproductive capacity, estrus cyclicity or sperm parameters. No effects on ED-relevant organ weights or histopathology in any group.		

No human data are available.

No other data are available.

A3.10.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The 2-generation study has been obtained through a letter of access to address this endpoint (Table A. 32, Anonymous 27, 2003 & Anonymous 28, 2007 (LoA)). This rat oral (drinking water) study was used to evaluate the effects of N-MIT on fertility. F0 males and females received N-MIT from 70 days prior to pairing, then throughout mating, gestation and lactation of two litters (F1 and F2). Animals were dosed with 0 ppm, 50 ppm (4-7 mg/kg bw/d M, 6-13 mg/kg bw/d F), 200 ppm (15-19 mg/kg bw/d M, 22-26 mg/kg bw/d F) and 1000 ppm N-MIT (69-86 mg/kg bw/d M, 93-115 mg/kg bw/d F). This treatment schedule was repeated for 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 treatment. There were no treatment-related effects on mean body weights or body weight gains prior to breeding at dose concentrations of 50 and 200 ppm. Decreased body weights were noted in the 1000 ppm group males and females. Test article-related effects were noted in the 1000 ppm group and consisted of: (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). Detailed data recorded for body weight gain are summarized below.

			Control		**Low dose – 50 ppm**		**Medium dose 200 ppm**		**Medium-high dose 1000 ppm**	
Body weight gain		**Generation**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
week 0-10	% of control	F0	--	--	99.6	94	100	94	84*	80*
week 18-36	% of control	F1	--	--	101	106	95	100	88*	99
gestation week 0-20	% of control	F0	--	--	--	102	--	98	--	86*
gestation week 0-20	% of control	F1	--	--	--	105	--	97	--	86*
lactation week 0-21	% of control	F0	--	--	--	107	--	85	--	137*
lactation week 0-21	% of control	F1	--	--	--	87	--	107	--	100

* = $p < 0.05$

This finding was most likely associated with decreased water consumption. 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 F0 and F1 parental animals

at any dose. No test-related macroscopic or microscopic changes neither effects on mean organ weights of F0 or F1 were observed at any dose level. Decreased water consumption was noted in F1 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 F1 generation during the week following weaning (post-natal days 21-28) when the animals were housed by litter. Decreased water consumption was noted in F2 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. 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 F1 and F2 pups at any dose. In the 1000 ppm group of P1 pups delay in the mean day of acquisition of balanopreputial separation and of vaginal patency was observed. The mean day of acquisition of balanopreputial separation and vaginal patency were within the historical data range. However, these effects were not a direct result of treatment, but were related to a decrease in mean body weights of pups at day of acquisition. Anogenital distances for the F2 pups were unaffected by treatment article. No microscopic changes were observed in the brains of pups of either the F1 or F2 generation exposed to 1000 ppm N-MIT *in utero*, through nursing, during lactation or in the drinking water following weaning. No evidence of adverse effects on reproductive performance was observed at doses up to and including 69-115 mg/kg/d (highest doses tested). Methylisothiazolinone is not a reproductive toxicant.
NOAEL for parental: 200 ppm [equiv. ♂/♀: 15/22 mg/kg bw/d] (based on reduced body weight and food consumption)

NOAEL reproductive toxicity 1000 ppm [equiv. ♂/♀: 69/93 mg/kg bw/d] (highest dose tested)

NOAEL for offspring toxicity: 200 ppm [equiv. ♂/♀: 15/22 mg/kg bw/d] (based on reduced body weight)

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. 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 test 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 F0 and F1 parental animals at any dose. No test-related macroscopic or microscopic changes neither effect on mean organ weights of F0 or F1 were observed at any dose.

No microscopic changes were observed in the brains of pups of either the F1 or F2 generation exposed to 1000 ppm N-MIT *in utero*, through nursing, during lactation or in the drinking water following weaning.

<u>Read across:</u>

Read-across to data generated using N-MIT is proposed for this endpoint (reproductive toxicity). The read-across is justified on the basis that the target substance (N-MIT·HCl) will dissociate to form N-MIT (the source substance) and HCl in the aqueous environment of the stomach following oral dosing. The source and target substances are therefore identical. The HCl generated from the dissociation of N-MIT·HCl is indistinguishable from the HCl produced by the stomach in significant quantities. HCl further dissociates in aqueous conditions

to the hydrogen and chloride ions, both of which are physiologically ubiquitous and subject to homeostatic control. The systemic toxicity of N-MIT·HCl and N-MIT will therefore be identical. While it is theoretically possible that the local effects of N-MIT·HCl on the stomach may be more marked as a consequence of dissociation of the molecule to form HCl, this would appear to be unlikely based on the relatively low dose levels used in the studies and the normal presence of quantities of HCl in the stomach. In any case, a more severe local effect produced by N-MIT·HCl would further limit the dose levels used in the studies, resulting in a lower level of systemic exposure to N-MIT. In this respect, the use of data generated using N-MIT is adequate for the reproductive toxicity endpoint.

A3.10.1.2 Comparison with the CLP criteria

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1A is reserved for substances known to be reproductive toxicants in humans. Since there is no evidence of N-MIT·HCl having caused reproductive toxicity in humans, classification in Category 1A is not justified.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be reproductive toxicants in humans and is largely based on data from animal studies where there is clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or not as a secondary non-specific consequence of other toxic effects. There is insufficient evidence of an effect of N-MIT reproductive performance and fertility from the available multi-generation study and repeated dose toxicity studies. Therefore, classification of N-MIT / N-MIT·HCl for fertility in Category 1B is not justified.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 2 is reserved for substances where there is some evidence from experimental animals of an adverse effect on sexual function and fertility but where the evidence is not sufficiently convincing to place the substance in Category 1. Any effects should be in the absence of other toxic effects, or not as a secondary non-specific consequence of other toxic effects. The available evidence shows that N-MIT no effects on reproductive performance and fertility. Therefore, classification of N-MIT / N-MIT·HCl for fertility in Category 2 is not justified.

In the two-generation reproductive toxicity study with N-MIT, there were no effects of treatment on fertility or reproductive capacity, estrus cyclicity or sperm parameters at dose levels sufficient to cause parental toxicity (reduced weight gain by up to 14%, reduced food intake by up to 125, reduced water intake by up to 41%). Slightly delayed sexual maturation in male F1 offspring (preputial separation attained at Day 46.9 compared to 44.4 days in controls) and in F1 female offspring (vaginal patency attained at Day 35.6 compared to 33.0 days in controls) was seen at the highest dose level. Effects were secondary to body weight effects; there were no effects of treatment on the body weight at attainment of sexual maturation in either sex as values were within the background range. Furthermore, there were no effects of treatment on anogenital distance in F2 pups. In the absence of any effects on sexual function or fertility, N-MIT does not meet the CLP classification criteria for any category.

A3.10.1.3 Conclusion on classification and labelling for effects on sexual function and fertility

No classification is required according to the criteria of Regulation (EC) 1272/2008.

A3.10.1.4 Overall conclusion on sexual function and fertility related to risk assessment

Not applicable for the CLH report.

A.3.10.2. Developmental toxicity

Table A.33 Summary table of animal studies on adverse effects on development

Summary table of animal studies on adverse effects on development							
Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/ group	Test substance (including purity), Vehicle, Dose levels,	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
OECD 414, GD 6-15, Oral (gavage), GLP, Reliability: 1	Rat, Sprague -Dawley, F, 25/gp	Kordek™ 573F (51.4% N-MIT; purity: 96-98%) 0, 5, 20, 60/40 mg/kg bw/d	NOAEL: Maternal: 20 mg/kg bw/d Developmental: 40 mg/kg bw/d	**60/40 mg/kg bw/d**: deaths (5/25) GD8-15; clinical signs in decedents (rocking, lurching or swaying, hypoactivity, rales, gasping, laboured breathing, decreased defecation, red material around the	No effects on foetal development	N-MIT	Anonymous 39, 2003 (LoA)

				nose, mouth and/or eyes) and in surviving females (rales, gasping and laboured breathing); reduced weight gain GD6-20 (-18%), reduced net weight gain (-28%); reduced food consumption GD 6-9 (-17%) discoloration of the stomach and lungs at necropsy. No fetal effects **20 mg/kg bw/d**: no maternal effects, no fetal effects **5 mg/kg bw/d**: no maternal effects, no fetal effects			
OECD 414, GD 6-28, Oral (gavage), GLP, Reliability: 1	Rabbit, New Zealand White, F, 25/group	Kordek™ 573F (51.4% N-MIT; purity: 96-98%)) 0, 3, 10, 30 mg/kg bw/d	NOAEL: Maternal: 10 mg/kg bw/d Developmental: 30 mg/kg bw/d	**30 mg/kg bw/d:** weight loss GD 6-9; reduced food consumption GD 6-9 (-19%), GD 9-12 (-11%); reduced defecation (4/25) GD7 onwards. Abortion (1/25) GD25. Dark areas of the stomach at necropsy (6/25). No fetal effects **10 mg/kg bw/d:** no maternal effects, no fetal effects **3 mg/kg bw/d:**	No effects on foetal development	N-MIT	Anonymous 26, 2003 (LoA)

				no maternal effects, no fetal effects			
OECD 414, GD 6-15, Oral (gavage), GLP, Reliability: 1	Rat/Crl (WI)BR, F, 25/group	Acticide SR 3267 (50% N-MIT); 0, 67, 100, 150 mg/kg bw/d (0, 33.4, 50, 75 mg/kg bw/d N-MIT)	NOAEL: Maternal: 33.4 mg/kg bw/d Developmental: 33.4 mg/kg bw/d	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	Fetal effects associated with maternal toxicity	N-MIT	Anonymous 40, 2002 (in CLH Report for MIT, 2015)

No human data are available.

No other data are available.

A3.10.2.1 Short summary and overall relevance of the provided information on adverse effects on development

Rat

In one developmental toxicity study in the rat, mated females received N-MIT by gavage from gestation days (GD) 6-19 at dose levels of 0, 5, 20 and 60/40 mg/kg bw/d (Table A.33, Anonymous 39, 2003). On GD6-9, the majority of high dose animals received 60 mg/kg bw/d. This dose exceeded the maximum tolerated dose and therefore the dose was lowered to 40 mg/kg bw/d. Three animals in the high dose group were found dead, and a further two rats were euthanized in a moribund state. Clinical signs in females either found dead or killed in extremis included rocking, lurching or swaying while ambulating, hypoactivity, rales, gasping, laboured breathing, decreased defecation, red material around the nose, mouth and/or eyes. In survivors, rales, gasping and laboured breathing 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 treatment-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/kg bw/d groups. N-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/d was determined, based on mortality, clinical signs, reduced body weight gain and necropsy findings at 40 mg/kg bw/d. The NOAEL for developmental toxicity was 40 mg/kg bw/day; the highest dose tested. Maternal toxicity: A test article-related lack of mean body weight gain (gestation days 6-9) and reductions in mean net body weight (not statistically significant), net body weight gain and food consumption (gestation days 6-9) were noted in the 60/40 mg a.i./kg/day group. The majority of the animals in this group were dosed at 60 mg a.i./kg/day during gestation days 6-9; after the dose level was reduced to 40 mg a.i./kg/day, no effects on body weight gain or food consumption were observed. Mean gravid uterine weight for the 60/40 mg a.i./kg/day group was unaffected by test article administration. No test article-related effects on mean body weight, body weight gain, net body weight, net body weight change, gravid uterine weight, and food consumption were noted in the 5 and 20 mg a.i./kg/day groups.At the scheduled necropsy on gestation day 20, no test-article related internal findings were observed at any dose level. Teratogenic data are summarized below:

Maternal effects

Parameter	**Control data**		**Low dose 5 mg a.i./kg/ day**	**Mid dose 20 mg a.i./kg/ day**	**High dose 60/40 mg a.i./kg/ day**	**Dose response +/-**
	Historical	**Study**				
Number of dams examined	1162	25	25	25	20	-

Clinical findings during application of test substance	NA	NA	NA	NA	inc.	-
Mortality of dams state %	2 %	0 %	0 %	0 %	20 %	-
Abortions	0	0	0	0	0	NA
Body weight gain (means) (g) day 0 -6	NA	32	31	29	31	NA
Body weight gain (means) (g) day 6-20	NA	114	107	109	94*	-
Body weight gain (means) (g) day 0-20	NA	146	137	138	125	-
Net Body weight change (means) (g)	NA	64.7	61.9	61.1	46.3 *	-
Food consumption (means) day 0 – 6	NA	23	22	23	23	NA
Food consumption (means) day 6 – 9	NA	24	24	23	20 *	NA
Food consumption (means) day 6 – 20	NA	25	25	25	23	NA
Food consumption (means) day 0-20	NA	25	24	24	23	NA
Water consumption	TS not applied in drinking water					
Pregnancies pregnancy rate or %	96.3%	100%	100%	96%	92%	-
Necropsy findings in dams dead before end of test	NA	NA	NA	NA	red areas in the glandular portion of the stomach and lung findings	

NA = not applicable

Intrauterine growth and survival were unaffected by test article administration. The number of fetuses (litters) available for morphological evaluation were 374 (25), 336 (25), 348 (24), and 284 (19) in the control, 5, 20 and 60/40 mg a.i./kg/day groups, respectively. Fetal external, visceral, or skeletal malformations were observed in 3 (3) and 1 (1) fetuses (litters) in the control and 60/40 mg a.i./kg/day groups, respectively. All malformations and developmental variations in this study were considered spontaneous in origin. There were no treatment-related effects on the numbers of early or late resorptions, live fetuses per litter, 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. Teratogenic effects are summarized below.

Litter response data

Parameter	Control data		Low dose 5 mg a.i./kg/ day	Medium dose 20 mg a.i./kg/ day	High dose 60/40 mg a.i./kg/ day	Dose response +/-
	Historical	Control				
External malformations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	0/0	0/0	0/0	NA
External variations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	0/0	0/0	0/0	NA
Visceral malformations [Nr. affected fetuses/Nr. affected litters]	NA	3/3	0/0	0/0	1/1	NA
Visceral variations [Nr. affected fetuses/Nr. affected litters]	NA	1/1	0/0	0/0	1/1	NA
Skeletal malformations [Nr. affected fetuses/Nr. affected litters]	NA	1/1	0/0	0/0	0/0	NA
Skeletal variations % [Nr. affected fetuses/Nr. affected litters]	NA	50.3%	56.8%	50.2%	53.1%	NA

NA = not applicable

Examination of foetuses

Parameter	Control data		Low dose 5 mg a.i./kg/ day	Medium dose 20 mg a.i./kg/ day	High dose 60/40 mg a.i./kg/ day	Dose response +/-
	Historical	Control				
External malformations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	0/0	0/0	0/0	NA

External variations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	0/0	0/0	0/0	NA
Visceral malformations [Nr. affected fetuses/Nr. affected litters]	NA	3/3	0/0	0/0	1/1	NA
Visceral variations [Nr. affected fetuses/Nr. affected litters]	NA	1/1	0/0	0/0	1/1	NA
Skeletal malformations [Nr. affected fetuses/Nr. affected litters]	NA	1/1	0/0	0/0	0/0	NA
Skeletal variations % [Nr. affected fetuses/Nr. affected litters]	NA	50.3%	56.8%	50.2%	53.1%	NA

NA = not applicable

In a further developmental toxicity study in the rat, mated females were gavaged with 0, 67, 100 and 150 mg/kg bw/d Acticide SR 3267, corresponding to 0, 33.4, 49.8 and 75 mg/kg bw/d N-MIT, from gestation days (GD) 6-15 (Table A.33, Anonymous 26, 2003). There were no maternal deaths or clinical signs attributed to treatment. Body weight gain of dams was significantly and dose-dependently reduced in animals treated with 100 and 150 mg/kg bw/d. In these groups, food consumption also decreased significantly. Body weight gain during the post-treatment period and total body weight gains during the pregnancy were similar in all groups. There were no necropsy findings related to treatment in any dose group. There were no significant differences in the number of the corpora lutea, implantations or viable foetuses; and no effects on numbers of 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. Foetal visceral examination revealed a significant increase in the number of the minor anomaly, dilated cerebral ventricles, at 150 mg/kg bw/d. The fetal skeletal examination revealed a significant increase in the number of unossified cervical vertebral bodies at 100 mg/kg (76% fetuses, 20/21 litters) and 150 mg/kg bw/d (72% foetuses, 21/22 litters). The number of unossified metatarsals was significantly higher at 150 mg/kg bw/d (78% foetuses, 21/22 litters). Delayed fetal ossification is related to decreased maternal body weight gain. Significant differences were detected between the control group and low dose group in the incidence of supernumerary rib without biological significance. In this study, a LOAEL of 50 mg/kg bw/d and of NOAEL 33.4 mg/kg bw/d were derived for developmental effects based on the increased incidence of unossified cervical vertebral bodies. A LOAEL of 50 mg N-MIT/ kg bw/d and a NOAEL of 33.4 mg/kg bw/d was derived for maternal toxicity based on decreased body weight gain during gestation. Data are summarized below:

Maternal toxicity:		
Overall	**Dosage** (mg MIT/kg bw/day)	**Mean body weight gain**
↓ food consumption was noted during	75	↓ (30 %)

treatment.	50		↓ (16%)	
Foetal toxicity:				
Overall	**Dosage** (mg MIT/kg bw/day)	**Incidence of dilated cerebral ventricles**	**Unossified metatarsals**	**Unossified cervical vertebral bodies**
No effects on intrauterine growth and survival, number of fetuses/litter, number of resorptions, fetal body weight or sex ratio.	75 mg MIT/kg bw/day:	12.3 % fetuses 14/22 litters	78 % foetuses, 21/22 litters	72 %, foetuses, 21/22 litters
	50 mg MIT/kg bw/day	0	0	78 % foetuses, 21/21 litters
	33.4 mg MIT/kg bw/day	no effect		

Rabbit

In rabbit pre-natal developmental toxicity study, N-MIT was administered on gestation days (GD) 6-28, the maternal animals were sacrificed on GD29 (Table A.33, Anonymous 26, 2003 (LoA)). One dam in the high dose group (30 mg/kg bw/d) aborted on GD25 and in the mid-dose group (10 mg/kg bw/d) one dam was found dead on GD, likely due to intubation error. At 30 mg/kg bw/d the following treatment-related effects were observed: decreased defecation (4/25, beginning on GD7), dark red areas in the stomach (6/25), mean body weight loss during GD6-9, reduced mean food consumption during GD6-9, 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. The single abortion reported at the high dose level was within the background range, and was not therefore considered to be related to treatment. 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. No treatment-related external malformations or developmental variations were noted at any dose level. No evidence of developmental toxicity was observed at doses up to and including 30 mg/kg bw/d, a dose level sufficient to cause maternal toxicity. Based on the results of this study, 10 mg/kg bw/d was considered to be the NOAEL for maternal toxicity; 30 mg/kg bw/d was considered to be the NOAEL for developmental toxicity. Below are detailed data:

Maternal effects

Parameter	Control data		Low dose 3 mg a.i./kg / day	Mid dose 10 mg a.i./kg / day	High dose 30 mg a.i./kg/ day	Dose response +/-
	Historical	Study				
Number of dams examined	1604	25	25	24	24	-
Clinical findings during application of test substance	NA	NA	NA	NA	dec.	+
Mortality of dams state %	1.3	0	0	4	0	-
Abortions	21	0	0	0	1	-
Body weight gain (means) (g) day 0 -6	NA	123	102	89	105	-
Body weight gain (means) (g) day 6-9	NA	20	7	11	23	-
Body weight gain (means) (g) day 9-12	NA	52	32	44	33	-
Body weight gain (means) (g) day 12 – 21	NA	144	131	92	136	-
Body weight gain (means) (g) day 21 – 29	NA	156	140	155	123	-
Net Body weight change (means) (g)	NA	90.2	-13.9	-23.6	33.0	-
Food consumption (means) day 0 – 6 (g/animal/day)	NA	150	150	150	147	-
Food consumption (means) day 6 – 9 (g/animal/day)	NA	150	149	147	121 *	+
Food consumption (means) day 9 – 12 (g/animal/day)	NA	149	148	145	132 *	+
Food consumption (means) day 12 – 21 (g/animal/day)	NA	148	144	133	132	-
Food consumption (means) day 21 – 29 (g/animal/day)	NA	138	137	134	130	-
Water consumption	TS not applied in drinking water					
Pregnancies pregnancy rate or %	86.6 %	84 %	84 %	72 %	80 %	-
Necropsy findings in dams dead before end of test	NA	NA	NA	NA	NA	-

Teratogenic effects

Litter response (Caesarean section data)

Parameter	Control data		Low dose 3 mg a.i./kg/ day	Mid dose 10 mg a.i./kg/ day	High dose 30 mg a.i./kg/ day	Dose response +/-
	Historical	Study				
Corpora lutea state total/number of dams	NA	210/25	236/25	169/24	200/24	-
Implantations state total/number of dams	NA	141/25	150/25	128/24	130/24	-
Resorptions state total/number of dams	NA	4/25	14/25	9/24	16/24	-
Total number of fetuses	8936	137	136	119	114	-
Pre-implantation loss *state %*	31.0	33.3	32.8	28.8	34.5	-
Post-implantation loss *state %*	9.0	2.5	9.7	10.3	10.7	-
Total number of litters	1389	21	21	19	21	-
Fetuses / litter	6.6	6.5	6.5	6.3	5.4	-
Live fetuses / litter *state ratio*	6.6	6.5	6.5	6.3	5.4	-
Dead fetuses / litter *state ratio*	0.0	0	0	0	0	-
Fetus weight (mean) [g]	46.8	46.8	47.8	45.9	46.5	-
Gravid uterus weight (mean) [g]	416.2	405.8	425.6	417.9	378.9	-
Crown-rump length (mean) [mm]	Not described in report					
Fetal sex ratio [% male]	1.01 (m/f)	44.0	46.9	45.4	41.8	-

Examinations of foetuses

Parameter	Control data Historical	Control data Study	Low dose 3 mg a.i./kg/ day	Medium dose 10 mg a.i./kg/ day	High dose 30 mg a.i./kg/ day	Dose response +/-
External malformations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	2/2	1/1	0/0	-
External variations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	0/0	0/0	0/0	-
Visceral malformations [Nr. affected fetuses/Nr. affected litters]	NA	1/1	2/2	2/2	0/0	-
Visceral variations [Nr. affected fetuses/Nr. affected litters]	NA	30/16	29/20	32/19	16/12	-
Skeletal malformations [Nr. affected fetuses/Nr. affected litters]	NA	0/0	3/3	4/3	0/0	-
Skeletal variations % [Nr. affected fetuses/Nr. affected litters]	NA	133/50	142/52	116/42	99/41	-

Read across:

Read-across to data generated using N-MIT is proposed for this endpoint (developmental toxicity). The read-across is justified on the basis that the target substance (N-MIT·HCl) will dissociate to form N-MIT (the source substance) and HCl in the aqueous environment of the stomach following oral dosing. The source and target substances are therefore identical. The HCl generated from the dissociation of N-MIT·HCl is indistinguishable from the HCl produced by the stomach in significant quantities. HCl further dissociates in aqueous conditions to the hydrogen and chloride ions, both of which are physiologically ubiquitous and subject to homeostatic control. The systemic toxicity of N-MIT·HCl and N-MIT will therefore be identical. While it is theoretically possible that the local effects of N-MIT·HCl on the stomach may be

more marked as a consequence of dissociation of the molecule to form HCl, this would appear to be unlikely based on the relatively low dose levels used in the studies and the normal presence of quantities of HCl in the stomach. In any case, a more severe local effect produced by N-MIT·HCl would further limit the dose levels used in the studies, resulting in a lower level of systemic exposure to N-MIT. In this respect, the use of data generated using N-MIT is adequate for the developmental toxicity endpoint.

A3.10.2.2 Comparison with the CLP criteria

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1A is reserved for substances known to be developmental toxicants in humans. Since there is no evidence of N-MIT / N-MIT·HCl having caused developmental toxicity in humans, classification in Category 1A is not justified.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be developmental toxicants in humans and is largely based on data from animal studies where there is clear evidence of an adverse effect on development in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects. N-MIT is shown not to be a developmental toxicant in studies in rats or rabbits at doses sufficient to cause maternal toxicity. There is insufficient evidence of an effect of N-MIT / N-MIT·HCl causing developmental toxicity in animals, classification in Category 1B is not justified.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 2 is reserved for substances where there is some evidence from experimental animals of an adverse effect on development but where the evidence is not sufficiently convincing to place the substance in Category 1. Any effects should be in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects. The available evidence shows that N-MIT has no effects on foetal development. Therefore, classification of N-MIT / N-MIT·HCl for developmental toxicity in Category 2 is not justified.

A3.10.2.3 Conclusion on classification and labelling for developmental toxicity

No classification is required according to the criteria of Regulation (EC) 1272/2008 .

A3.10.2.4 Overall conclusion on effects on development related to risk assessment

Not applicable for the CLH report.

A.3.10.3. Effects on or via lactation

No available data.

A3.10.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

Refer to reproductive toxicity study

A3.10.1.2 Comparison with the CLP criteria
No classification is required according to the criteria of Regulation (EC) 1272/2008

A3.10.3.3 Overall conclusion on effects on or via lactation related to risk assessment

Not applicable for the CLH report.

A3.10.4 Conclusion on classification and labelling for reproductive toxicity

N-MIT does not meet the CLP criteria for classification for effects on sexual function and fertility, developmental toxicity, or effects on or via lactation.

A3.10.5 Overall conclusion on reproductive toxicity related to risk assessment

Not applicable for the CLH report.

A.3.11. Aspiration hazard

No data are available.

A3.11.1 Short summary and overall relevance of the provided information on aspiration hazard

No data has been generated. N-MIT·HCl is neither a hydrocarbon with a kinematic viscosity cut-off ≤20.5 mm nor is there available good reliable human evidence of the human aspiration toxicity hazards.

A3.11.2 Comparison with the CLP criteria

N-MIT·HCl is neither a hydrocarbon with a kinematic viscosity cut-off ≤20.5 mm nor is there available good reliable human evidence of the

human aspiration toxicity hazards.

A3.11.3 Conclusion on classification and labelling for aspiration hazard

No classification is required according to the criteria of Regulation (EC) 1272/2008

A.3.12. Neurotoxicity

A3.12.3 Conclusion on neurotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.13. Immunotoxicity

A3.13.3 Conclusion on immunotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.14. Endocrine disruption

Not applicable to CLH report.

A.3.15. Further Human data

No further human data are available.

A.3.16. Other data

No other data are available.

A.4. Environmental effects assessment

N-MIT·HCl dissociates in contact with water and become (de-) protonated according to the pH of the solution. Under typical use conditions, in which the active substance is highly diluted (e.g. maximum in-use concentration: 0.999 % w/w) the protonated form of the active substance will not depend on whether N-MIT·HCl or N-MIT (free base) was used as biocidal product. An application for approval of 2-methyl-2,3-dihydro-1,2-thiazol-3-one (N-MIT) has already been submitted as a PT 6 biocidal active substance. Consequently, the read across has been undertaken using the ECHA Read-Across Assessment Framework (RAAF, 2017a) from the already submitted and approved N-MIT (free base) to the new active substance N-MIT·HCl. Please refer to Document with a justification for the read across available in confidential Annex II (Appendix VIII: Read across between N-MIT·HCl and N-MIT).

Please note that the data provided in italics are based on studies performed for the active substance N-MIT and have been taken from the MIT CAR for PT 13 (November 2014). A letter of access (LoA) to the N-MIT, PT 6 studies owned by SEMS GmbH, a wholly-owned subsidiary of DuPont de Nemours Inc. has been submitted. For more details for hazardous properties of N-MIT, please refer to the MIT PT 11 Assessment Report (January 2017).

A.4.1. Fate and distribution in the environment

A.4.1.1. Degradation

A4.1.1.1 Abiotic degradation

Hydrolysis

Table A.34 Summary table - Hydrolysis

Summary table - Hydrolysis							
Guideline / Test method, Reliability	pH	Temp. [°C]	Initial test substance conc. [µg/mL]	Reaction rate constant, k [h]	Half-life [d]	Coefficient of correlation, r^2	Reference
US EPA N161-1 *RI=1*	*5* *7* *9*	*23.5-26*	*13.3* *9.9* *10.1*	*ND (stable)*	*ND (stable)*	*N/A*	*Anonymous, 1992 (Rohm and Haas) (LoA)*

Phototransformation in water

Table A.21 Summary table – Photolysis in water

Summary table – Photolysis in water							
Guideline / Test method, Reliability	Initial test substance conc. [µg/mL]	Total recovery of test substance [% of appl. AS]	Photolysis rate constant (kcp) [d^{-1}]	Direct photolysis sunlight rate constant (kpE)	Reaction quantum yield (φcE)	Half-life (t1/2E) [d]	Reference
US EPA N161-2 *RI=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*	*Anonymous, 1995* *(Rohm and Haas)* *(LoA)*

Estimated photo-oxidation in air

Table A.22 Summary table – Photo-oxidation in air

Summary table – Photo-oxidation in air					
Model	Light protection (yes/no)	Estimated daily (24h) OH concentration [OH/cm^3]	Overall OH rate constant [cm^3/molecule sec]	Half-life [hr]	Reference
AOPWIN™ RI=2	NA	5E+05	26.84E-12	14.35	*Anonymous*, 2018 (Roche)

A4.1.1.2 Biotic degradation

A4.1.1.2.1 Biodegradability (ready/inherent)

Table A.37 Summary table - biodegradation studies (ready/inherent):

Summary table - biodegradation studies (ready/inherent)								
Guideline /	Test type	Test	Inoculum	Additional	Test	Degradation	Remarks	Reference

Test method, Reliability		parameter	Type	Conc.	Adaptation	substrate	substance conc.	Incubation period	Degree [%]	[positive control]	
OECD 301B, EC C.4-C, OCSPP 835.3110 RI=2	Ready biodegradability	CO_2	Activated sewage sludge	30 mg SS/L	No	No	31.6 mg/L (equivalent to 10 mg carbon/L)	28 days	0		*Anonymous*, 2017c (Roche)
OECD 301B, EEC C4-C Modified Sturm test *RI=1*	*Ready biodegradability* *Based on metabolite NMMA*	*CO_2*	*WWTP activated sludge*	*30 mg d.w./mL washed sludge*	*No*		*36*	*9* *28*	*80.9* *94.7*		*Anonymous, 2003a* *(Rohm and Haas)* *(LoA)*
OECD 301B, EEC C4-C Modified Sturm test *RI=1*	*Ready biodegradability* *Based on metabolite NMA*	*CO_2*	*WWTP activated sludge*	*30 mg d.w./mL washed sludge*	*No*		*30.2*	*9* *28*	*46.4* *78.2*		*Anonymous, 2003b* *(Rohm and Haas)* *(LoA)*
OECD 301B, EEC C4-C Modified Sturm test *RI=1*	*Ready biodegradability* *Based on metabolite MA*	*CO_2*	*WWTP activated sludge*	*30 mg d.w./mL washed sludge*	*No*		*43*	*9* *28*	*78.2* *91.9*		*Anonymous, 2003c* *(Rohm and Haas)* *(LoA)*

N-MIT·HCl: The ready biodegradability of the N-MIT·HCl was tested according to the OECD guideline 301B. The biodegradation of the test substance N-MIT·HCl was investigated at the test concentration of 10 mg carbon/L in sealed culture vessels inoculated with activated sewage-sludge. Results from test on ready biodegradability showed that N-MIT·HCl was not readily biodegradable as 0% biodegradation was attained after 28 days. 0% biodegradation was attained also in toxicity control thereby confirming that N-MIT·HCl exhibit an inhibitory effect on the sewage treatment microorganisms used in the test. In such instances, OECD guideline 301 proposes a lower test concentration. If inhibition due to toxic effects is to be avoided, it is suggested in Annex II of the guideline that the tested

concentration should be < 1/10 of the EC_{50} obtained in the activated sludge inhibition test that is < 0.23 mg/L. Despite toxic effects of the N-MIT·HCl were not investigated at lower concentration, no further testing is triggered since the biodegradation test result "not readily biodegradable" reflects the worst-case assumption for exposure assessment. Further, also N-MIT was shown as not readily biodegradable according to the MIT PT 11 Assessment Report (2017).

Metabolites: A letter of access is available for the ready biodegradability studies of the metabolites N-methyl malonamic acid (NMMA), N-methyl acetamide (NMA) and malonamic acid (MA). All three were determined to be readily biodegradable.

A4.1.1.3 Rate and route of degradation including identification of metabolites and degradation products

A4.1.1.3.1 Biological sewage treatment

Not applicable for CLH report

A4.1.1.3.2 Biodegradation in freshwater

Aerobic aquatic degradation

No experimentally derived data are available.

Water/sediment degradation test

No data are available: a waiver is proposed for this endpoint.

A4.1.1.3.3 Biodegradation in seawater

Seawater degradation study

No experimentally derived data are available.

Seawater/sediment degradation study

No data are available: a waiver is proposed for this endpoint.

A4.1.1.3.4 Higher tier degradation studies in water or sediment

No experimentally derived data are available, since the environmental risk assessment on N-MIT·HCl can be carried out based on representative laboratory data.

A4.1.1.3.5 Biodegradation during manure storage

No data are available: a waiver is proposed for this endpoint.

A4.1.1.3.6 Biotic degradation in soil

No experimentally derived data are available.

A4.1.1.3.7 Laboratory soil degradation studies

Aerobic biodegradation

No data are available: a waiver is proposed for this endpoint.

Anaerobic biodegradation

No data are available: a waiver is proposed for this endpoint.

A4.1.1.3.8 Higher tier degradation studies in soil

Field dissipation studies (field studies, two soil types)

No data are available: a waiver is proposed for this endpoint.

A4.1.1.3.9 Short summary and overall relevance of the provided information on degradation and conclusion on rapid degradation

The available hydrolysis study carried out according to the US EPA N161-1 guideline shows that no significant hydrolysis of N-MIT was observed at pH 5, 7 and 9. Therefore, N-MIT·HCl is considered to be hydrolytically stable at environmental conditions.

The photodegradation of N-MIT in water that was studied according to US EPA N161-2 guideline shown that N-MIT was photolytically degraded at a moderate rate with a half life of 11.1 days. Two major metabolites were produced: 3-methyl-4-thiazolin-2-one and N-methyl malonamic acid.

Results of ready biodegaradability test conducted according to the OECD guideline 301B showed that N-MIT·HCl was not readily biodegradable as 0% biodegradation was attained after 28 days. 0% biodegradation was attained also in toxicity control thereby confirming that N-MIT·HCl exhibit an inhibitory effect on the sewage treatment microorganisms used in the test. Moreover, also N-MIT was shown as not readily biodegradable according to the MIT PT 11 Assessment Report (2017).

Ready biodegradation studies on metabolites N-methyl malonamic acid (NMMA), malonamic acid (MA) and N-methyl acetamide (NMA) performed following OECD guideline 301B shown that all the three metabolites tested are readily biodegradable.

A.4.1.2. Distribution

A4.1.2.1 Adsorption onto/desorption from soils

Table A.38 Summary table – Adsorption/desorption

Summary table – Adsorption/desorption									
Guideline / Test method, Reliability	Soil class	Adsorbed AS [%]	K_a	K_{aOC}	K_d	K_{dOC}	K_a / K_d	Remarks	Reference
US EPA N163-1 RI=2	*Sandy loam*	*10.5*	*0.1*	*7.7*	*0.67*	*ND*	*0.015*		*Anonymous, 2006 (Rohm and Haas) (LoA)*
	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*		

K_a = Adsorption coefficient
K_{aOC} = Adsorption coefficient based on organic carbon content
K_d = Desorption coefficient
K_{dOC} = Desorption coefficient based on organic carbon content
K_a / K_d = Adsorption / Desorption distribution coefficient

The Koc value of N-MIT in five soils examined was 7.5 L/kg (arithmetic mean) in accordance with the MIT PT 11 Assessment Report (2017). The available study indicates N-MIT is considered highly mobile.

A4.1.2.2 Higher tier soil adsorption studies

No experimentally derived data are available, since the environmental risk assessment on N-MIT·HCl can be carried out based on representative laboratory data.

A4.1.2.3 Volatilisation

Regarding volatilisation, please see Part A, section 1.3 Physical and chemical properties of the active substance.

A.4.1.3. Bioaccumulation

Measured aquatic bioconcentration

No data are available: a waiver is proposed for this endpoint.

Estimated aquatic bioconcentration

Table A.39 Summary table – Estimated aquatic bioconcentration

Summary table – Estimated aquatic bioconcentration					
Basis for estimation	Log Kow (measured)	Estimated BCF for fish (freshwater)	Estimated BCF for fish eating bird/predator	Remarks	Reference
EPI Suite™	- 0.44	3.16 L/kg	Not determined		*Anonymous*, 2018 (Roche)

The log Kow based on EPI Suite™ estimation for N-MIT·HCl is - 0.44. As such, it can be considered that the active substance does not possess any bioconcentration potential as this value is several orders of magnitude lower than the CLP trigger value of 4. Likewise, also N-MIT exhibits negligible potential for bioaccumulation according to the N-MIT PT 11 Assessment Report (2017) having log Kow value of - 0.32 and BCFfish of 0.107 L/kg.

A.4.1.4. Monitoring data

No monitoring data are available.

A.4.2. Effects on environmental organisms

N-MIT·HCl dissociates in contact with water and become (de-) protonated according to the pH of the solution. Under typical use conditions, in which the active substance is highly diluted (e.g. maximum in-use concentration: 0.999 % w/w) the protonated form of the active substance will not depend on whether N-MIT·HCl or N-MIT (free base) was used as biocidal product. An application for approval of 2-methyl-2,3-dihydro-1,2-thiazol-3-one (N-MIT) has already been submitted as a PT 6 biocidal active substance. Consequently, the read across has been undertaken using the ECHA Read-Across Assessment Framework (RAAF, 2017a) from the already submitted and approved N-MIT (free base) to the new active substance N-MIT·HCl. Please refer to Document with a justification for the read across available in confidential Annex II (Appendix VIII: Read across between N-MIT·HCl and N-MIT).

Please note that data provided in italics are based on studies performed for the active substance N-MIT and have been taken from the MIT CAR for PT 13 (November 2014). In addition, new studies with N-MIT·HCl on acute toxicity to *Daphnia magna* (Anonymous, 2017a) and growth inhibition to algae (Anonymous, 2017b) are available.

A.4.2.1. Atmosphere

Not applicable for CLH report.

A.4.2.2. Toxicity to sewage treatment plant (STP) microorganisms

Inhibition of microbial activity (aquatic)

Table A.23 Summary table – inhibition of microbial activity

Summary table – inhibition of microbial activity							
Guideline / Test method, Reliability	Species/ Inoculum	Endpoint	Exposure		Results [mg a.i./L]		Reference
			Design	Duration [h]	EC_{10}	EC_{50} (nominal)	
OECD 209 *RI=1*	*Activated sludge*	*3h EC_{50}*	*Respiration inhibition*	*3*	-	*41*	*Anonymous, 1996 (Rohm and Haas) (LoA)*

Inhibition of microbial activity of N-MIT was tested in a 3-hour respiration inhibition test with activated sludge. The EC_{50} from the study is 41 mg a.i./L based on nominal concentrations.

A.4.2.3. Aquatic compartment

A4.2.3.1 Freshwater compartment

Acute/short-term toxicity (freshwater)

Table A.24 Summary table – acute/short-term aquatic toxicity

Summary table – acute/short-term aquatic toxicity									
Guideline / Test method, Reliability	Species	Test material	Endpoint	Exposure		Results			Reference
				Design	Duration [h]				
Fish									
No data available									
Invertebrates						**EC_{10}**	**EC_{20}**	**EC_{50}**	
OECD 202, EC C.2 RI=1	Water flea *Daphnia magna*	N-MIT·HCl (>99.9%)	48 h EC_{50}	Static	48	1.44 mg a.i./L, nominal	1.70 mg a.i./L, nominal	2.33 mg a.i./L, nominal	*Anonymous*, 2017a (Roche)
Algae (growth inhibition)						**E_rC_{10}**	**NOE_rC**	**E_rC_{50}**	
OECD 201, US EPA 122-2, EEC C.3 *RI=2*	*Freshwater algae Selenastrum capricornutum*	*N-MIT (97.8%)*	*120 h EC_{50} / NOEC*	*Static*	*120*	*0.062 mg a.i./L, initial measured (24 h)*	-	*0.102 mg a.i./L, initial measured (24 h)*	*Anonymous, 1997* *(Rohm and Haas)* *(LoA)*
OECD 201, EC C.3 RI=1	Freshwater green algae *Pseudokirchneriella subcapitata*	N-MIT·HCl (>99.9%)	72 h EC_{50} / NOEC	Static	72	0.208 mg a.i./L, mean measured (72 h)	0.047 mg a.i./L, mean measured (72 h)	0.289 mg a.i./L, mean measured (72 h)	*Anonymous*, 2017b (Roche)

Results from an acute static toxicity study with *Daphnia magna* indicate that N-MIT·HCl is toxic to freshwater invertebrates. The study was conducted under GLP and in line with the OECD 202 guideline and met its validity criteria. The nominal concentrations tested were 100, 45.5, 20.7, 9.4, 4.3, 1.9 and 0.9 mg test item/L and a control (20 female daphnids <24 hours old were tested per control

and test concentration, divided into 4 groups of 5 animals). Analytical determination was performed in samples taken on 0 and 48 hours and all reported results refer to nominal values since the concentrations of the test item were within ±20% of the nominal concentrations during the test. The 48 h EC_{50} from the study is 2.33 mg a.i./L based on nominal concentrations.

Two toxicity tests with the freshwater alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) are available for N-MIT and N-MIT·HCl.
A static 120 h toxicity test to *Selenastrum capricornutum* using N-MIT indicates that validity criteria (detailed in OECD 201 guideline) were met and the test is considered reliable. A 476-fold increase in control cultures was observed indicating exponential growth and multiple generations. In addition, mean coefficients of variation in the control cultures were 22.04 for day 0-1, 8.74 for day 1-2, 13.04 for day 2-3, 11.47 for day 3-4 and 7.43 for day 4-5 and the coefficient of variation of average specific growth rate during the whole test period in replicate control culture was calculated as 0.76. Results from the toxicity study indicate that the substance is very toxic to freshwater algae. In this study, the concentration of the test substance was not maintained at >80 % of nominal concentrations, due to fast biodegradation of N-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 N-MIT depends on the algal concentration (i.e. the concentration dependency can be attributed to the role of algae in the degradation of N-MIT). N-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 N-MIT by algae. At higher test concentrations toxic to algae, growth of algae is inhibited which in turn slows down the degradation of N-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 N-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 N-MIT. For this reason, the 24 hour E_rC_{10} based on initial measured concentrations was used as endpoint.

In a 72-hr study conducted under GLP with N-MIT·HCl as the test item on *Pseudokirchneriella subcapitata* in line with the OECD 201 guideline, the validity criteria were met (*i.e.* the increase in control cultures was 131-fold (>16-fold) within 72 hours, the coefficient of variation of sectional (daily) growth rates in control cultures was 8.4% (<35%) and the coefficient of variation of average growth between control replicates was 3.8% (<7%)). The nominal concentrations tested were 1.00, 0.32, 0.10, 0.032 and 0.01 mg test item/L and a control (three replicates per test concentration and six replicates in the control were used, while the test was started (0 hours) by inoculation of a biomass of approx. 5000 algal cells per mL test medium). Analytical determination was performed in samples taken on 0 and 72 hours. Decline of the test substance concentrationwas observed as in the samples taken on 0 hours the measured concentrations varied from 95% to 103% of nominal, while in the samples taken on 72 hours the measured concentrations varied from 14% to 100% of nominal.. Similarly as in the case of N-MIT, at higher test concentrations, degradation of test substance was slower in comparison with lower test concentrations. The endpoints were based on the geometric mean measured concentrations of the test item: 1.00, 0.28, 0.047, 0.012 and 0.004 mg test item/L. The E_rC_{50} and the EyC_{50} after 72 hours were determined to be 0.289 mg a.i./L and 0.112 mg ai./L, respectively. The 72-hr NOEC for both growth rate and yield was 0.047 mg a.i./L. It is noted that although both of the dose response curves for growth rates and yield were of good quality (monotonous) the EC_{10}, EC_{20} and EC_{50}

values for growth rate were accompanied by 95% confidence intervals (unbound low intervals and non-determined high intervals in all cases) that indicate some quality issues regarding their statistical robustness.
In the study, the E_rC_{50} after 24, 48 and 72 h following independent statistical analysis by eCA were determined to be 0.825, 0.249 and 0.290 mg a.i./L, respectively. These results illustrate that after 24 h decrease in concentration is accompanied by a decrease in growth inhibition and thereby confirming that recovery starts during the test.

Chronic/long-term toxicity (freshwater)

Table A.25 Summary table – chronic/long-term aquatic toxicity

Summary table – chronic/long-term aquatic toxicity								
Guideline / Test method, Reliability	Species	Test material	Endpoint	Exposure		Results		Reference
				Design	Duration [d]	NOEC	LOEC	
Fish								
OECD 210, US EPA OPPTS 850.1400, US EPA 72-4, US EPA 797.1600 *RI=1*	*Rainbow trout Oncorhynchus mykiss*	*N-MIT (as a formulated product, 51.252 % in water)*	*ELS NOEC*	*Flow-through*	*98*	*2.38 mg a.i./L, mean measured (growth, wet weight)*	*4.93 mg a.i./L, mean measured (growth, wet weight)*	*Anonymous, 2005 (Rohm and Haas) (LoA)*
Invertebrates								
OECD 211, US EPA OPPTS 850.1300 *RI=1*	*Water flea Daphnia magna*	*N-MIT (as a formulated product, 51.252 % in water)*	*21 days NOEC*	*Flow-through*	*21*	*0.0442 mg a.i./L, mean measured (dry weight)*	*0.0889 mg a.i./L, mean measured (dry weight)*	*Anonymous, 2004 (Rohm and Haas) (LoA)*

A letter of access is available for the chronic studies with fish and invertebrates.
A GLP early life stage chronic toxicity study with N-MIT on rainbow trout (*Oncorhynchus mykiss*) in line with the OECD 210 guideline is available. The study was conducted under flow-through conditions (for 98 days (62 days post-hatch)) and met the OECD 210 validity criteria. The nominal concentrations tested were 20, 10, 5, 2.5, 1.3, and 0.63 mg test item/L and a control (4 replicate vessels per treatment and 15 animals/vessel) with measured concentrations: < MQL (control), 0.613, 1.20, 2.38, 4.93, 9.88, and 20.0 mg

test item/L. The $NOEC_{growth}$ was determined to be 2.38 mg a.i./L.
A GLP chronic toxicity study with N-MIT on *Daphnia magna* in line with the OECD 211 guideline is available. The study was conducted under flow-through conditions (for 21 days) and met all the OECD 210 validity criteria. The nominal concentrations tested were 0.4, 0.2, 0.1, 0.05, 0.025, and 0.013 mg test item/L and a control (4 replicate vessels per treatment and 10 animals (< 24 hours old)/vessel) with measured concentrations: 0.0117, 0.0209, 0.0442, 0.0889, 0.183 and 0.359 mg a.i./L mg test item/L (mean values from analyzed samples taken on days 0, 7, 14 and 21). The lowest chronic value from this study is the 21-day $NOEC_{growth}$ of 0.0442 mg a.i./L, based on significant effects on dry weight at the next concentration level. However, it should be noted that growth is an optional test parameter according to the OECD guideline 111.

A4.2.3.2 Sediment compartment

No data are available: a waiver is proposed for this endpoint.

A4.2.3.3 Marine compartment

No data are available: a waiver is proposed for this endpoint.

A4.2.3.4 Sea sediment compartment

No data are available: a waiver is proposed for this endpoint.

A4.2.3.5 Higher tier studies on aquatic organisms

No experimentally derived data are available, since the environmental risk assessment on N-MIT·HCl can be carried out based on the available data.

A.4.2.4. Terrestrial compartment

Not applicable for CLH report.

A.4.2.5. Groundwater

Not applicable for CLH report.

A.4.2.6. Birds and mammals

Not applicable for CLH report.

A.4.2.7. Primary and secondary poisoning

Not applicable for CLH report.

A.4.3. Endocrine disruption

Not applicable for CLH report.

A.4.4. Derivation of PNECs

Not applicable for CLH report.

A.4.5. Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

A.4.5.1. Short-term (acute) aquatic hazard

Table A.26 Summary of key information on acute/ short-term aquatic toxicity relevant for acute classification

Method, Reliability	Species	Test material	Results	Remarks	Reference
Invertebrates					
OECD 202, EC C.2 RI=1	*Daphnia magna*	N-MIT·HCl, purity: >99.9%	48 h EC_{50} = 2.33 mg a.i./L, nominal		*Anonymous*, 2017a (Roche)
Algae					
OECD 201, EC C.3 Static RI=1	*Pseudokierchneriella subcapitata*	N-MIT·HCl, purity: >99.9%	72 h E_rC_{50} = 0.289 mg a.i./L, mean measured		*Anonymous*, 2017b (Roche)

In the acute aquatic tests, algae were found to be the most sensitive trophic level based on the available data with N-MIT and N-MIT·HCl, with all derived E_rC_{50} values <1 mg/L. The endpoint from the study owned by Roche (Anonymous, 2017b) is selected over the endpoint from the study owned by Rohm and Haas (Anonymous, 1997) as 1) the test item is the actual substance being classified *i.e.* N-MIT·HCl and it should supersede read-across data and 2) as the endpoint from this study is a 72-hr E_rC_{50} value which is well justified to be used (at these levels the concentration in the test was maintained well at the static system (98% on 0 hours and 78% on 72 hours of the nominal concentration of 0.32 mg test item/L) while the lower endpoint from the study with N-MIT is a 24-hr E_rC_{50} endpoint which is not the appropriate time-scale and it was based in initial measured values as a precautionary approach. Therefore, it is concluded that N-MIT·HCl fulfils the criteria for classification as Acute aquatic Category 1, H400 (Very toxic to aquatic life) with an M-factor of 1, based on the N-MIT·HCl 72 h E_rC_{50} in the range $0.1 < L(E)C_{50} \leq 1.0$.

A.4.5.2. Chronic/ long-term aquatic hazard (including information on bioaccumulation and degradation)

Table A.27 Summary of key information on chronic/ long-term aquatic toxicity relevant for chronic classification

Method	Species	Test material	Results	Remarks	Reference
Invertebrates					
OECD 211, US EPA OPPTS 850.1300 *Flow-through* *RI=1*	*Daphnia magna*	*N-MIT*	*21 d NOEC = 0.0442 mg a.i./L, mean measured (dry weight)*		*Anonymous, 2004* *(Rohm and Haas)*
Algae					
OECD 201, EC C.3 Static RI=1	*Pseudokirchneriella subcapitata*	N-MIT·HCl	72 h NOE_rC= 0.047 mg a.i./L, mean measured		*Anonymous*, 2017b (Roche)

To compare endpoints with N-MIT·HCl to endpoints for N-MIT a correction for molar mass of N-MIT to N-MIT·HCl should be applied. The algal NOE_rC for N-MIT is higher, if corrected for difference in molar mass as 0.062 mg N-MIT/L corresponds to 0.082 mg N-MIT·HCl/L. Similarly, when converted, the chronic toxicity NOEC for *Daphnia magna* of 0.0442 mg N-MIT/L is 0.058 mg N-MIT·HCl/L while the chronic fish endpoint is clearly higher (2.38 mg N-MIT/L). Considering these, algae were the most sensitive trophic group in the chronic toxicity studies with a 72 h NOE_rC of 0.047 mg N-MIT·HCl/L obtained in the freshwater alga *Pseudokierchneriella subcapitata* study. The lowest endpoint value for algae fulfils the criteria $NOEC/EC_x \leq 0.1$ mg/L. Being not rapidly degradable, N-MIT·HCl therefore fulfils criteria for classification as Chronic aquatic Category 1, H410 (Very toxic to aquatic organisms with long lasting effects) with an M-factor of 1 due to the NOE_rC in the range $0.01 < NOEC/EC_x \leq 0.1$.

A.4.5.3. Conclusion on classification and labelling for environmental hazards and comparison with the CLP criteria

Aquatic Acute 1, M-factor 1

Aquatic Chronic 1, M-factor 1

Pictogram code: GHS09

Hazard statement on the label: Very toxic to aquatic life with long lasting effects (H410)

A.5. Assessment of additional hazards

A.5.1. Hazardous to the ozone layer

A.5.1.1. Short summary and overall relevance of the provided information on ozone layer hazard

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

with ozone.

A.5.1.2. Comparison with the CLP criteria

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

A.6. Additional Labelling

According to CLP for substances in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled, the EUH071 – "Corrosive to the respiratory tract" is applicable. N-MIT·HCl is proposed to be classified as Skin Corr. 1A. N-MIT·HCl is in the form of powder. There is a possibility of exposure via inhalation, since the particle size distribution indicates that the proportion of particle sizes below 50 µm is 30%. N-MIT·HCl shall be labelled as EUH071.

D. Appendices

APPENDIX V: OVERALL REFERENCE LIST (INCLUDING DATA OWNER AND CONFIDENTIALITY CLAIM)

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner	Applicability	
						CAR	CLH
Physicochemical properties and evaluation of physical hazards							
Anonymous	2018	2.9-1	Manufacturing process of N-MIT·HCl (CAS 26172-54-3) X92019, Unpublished	Yes	Roche	Yes	Yes
Anonymous	2018	2.9-2	Specification sheet, Sigma-Aldrich, Published	No	Sigma-Aldrich	Yes	Yes
Anonymous	2018	2.9-3	N-MIT Safety Data Sheet (SDS), Sigma-Aldrich, Published	No	Sigma-Aldrich	Yes	Yes
Anonymous	2020	2.9-4	Manufacturing process of N-MIT·HCl (CAS 26172-54-3) in Buchs, Switzerland, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017a	3.1	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Physical State, Colour and Odour, ibacon GmbH, Report No. 123831203, GLP, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017b	3.2	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Melting Point / Melting Range, ibacon GmbH, Report No. 123831180, GLP, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017c	3.3-1	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the pH-Value, ibacon GmbH, Report No. 123831352, GLP, Unpublished	Yes	Roche	Yes	Yes
Fieseler, A.	2017a	3.3-2	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Acidity or Alkalinity, ibacon GmbH, Report No. 123831349, GLP, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017d	3.4	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Boiling Point, ibacon GmbH, Report No. 123831181, GLP, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017e	3.5	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Relative Density, ibacon GmbH, Report No. 123831182, GLP, Unpublished	Yes	Roche	Yes	Yes

Wagner Rivas, V.	2017f	3.7.1	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Vapour Pressure by Isothermal Thermogravimetry, ibacon GmbH, Report No. 123831183, GLP, Unpublished	Yes	Roche	Yes	Yes
Moseley, R.	2018	3.7.2	2-Methyl-4-Isothiazolin-3-One Hydrochloride, CAS Number 26172-54-3: Henry's law constant, ERM, Report No. 0372224-HL1, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017g	3.8	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Surface Tension, ibacon GmbH, Report No. 123831184, GLP, Unpublished	Yes	Roche	Yes	Yes
Iffland, D.	2017	3.9	Water Solubility of 2-Methyl-4-isothiazolin-3-one hydrochloride, Eurofins Agroscience Services EcoChem GmbH, Report No. S17-05445, GLP, Unpublished	Yes	Roche	Yes	Yes
Wöhr, T.	2018	3.10	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Coefficient (n-Octanol/Water) by the Shake Flask Method, ibacon GmbH, Report No. 123831186, GLP, Unpublished	Yes	Roche	Yes	Yes
Cihiy, J.S.	1995	3.11 (LoA)	Product chemistry series 63: physical and chemical characterization studies of Kordek(TM) 573T Industrial Microbiocide, Rohm and Haas Company, Report No. TR-95-31, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Fieseler, A.	2017b	3.13	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Dissociation Constant in Water, ibacon GmbH, Report No. 123831194, GLP, Unpublished	Yes	Roche	Yes	Yes
Ahrens, A.	2018	3.14	Particle Size Distribution, Siemens AG, Report No. PS20170413-1, GLP, Unpublished	Yes	Roche	Yes	Yes
Betteley, J.	2001	3.16 (LoA)	Kordek(TM) 573T industrial microbiocide physicochemical properties, Huntingdon Life Sciences Ltd., Report No. RAS 201/012606, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Wöhr, T.	2020	3.16	N-Methylisothiazolone HCl: Temperature-dependent Determination of the Solubility in Organic Solvents, Report No. 148081201, GLP, Unpublished	Yes	Roche	Yes	Yes
Gledhill, I.	2021	4.1	Differential scanning calorimetry (DSC) testing on a sample of N-MIT·HCL, DEKRA UK Ltd, Report No. GLP/3016010093, GLP, Unpublished	Yes	Roche	Yes	Yes

Wagner Rivas, V.	2017h	4.7	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Flammability, ibacon GmbH, Report No. 123831187, GLP, Unpublished	Yes	Roche	Yes	Yes
Gledhill, I.	2022	4.8	Heat Accumulation Storage Testing on a sample of N-MIT.HCL, DEKRA UK Ltd, Report No. GLP/3016010498, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous	2018	4.10, 4.12, 4.15, 4.16, 4.17.3, 5.2.2	Waivers, ERM, Report. No. 0372224, Unpublished	Yes	Roche	Yes	Yes
Roche	2010	4.8, 4.10, 4.12, 4.16	Safety Tests Data Sheet, Roche Basel, Report No. BS-9626, Unpublished	Yes	Roche	Yes	Yes
Wagner Rivas, V.	2017i	4.11, 4.17.2	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Determination of the Relative Self-Ignition Temperature, ibacon GmbH, Report No. 123831188, GLP, Unpublished	Yes	Roche	Yes	Yes
Comb, T.	2021	3.4.1-1	N-MIT·HCl: Accelerated Storage Stability, AgroChemex Environmental Ltd., Study number: ENV-20-363, GLP, Unpublished	Yes	Roche	Yes	Yes
Comb, T.	2020 2021	3.4.1-2 3.4.1-3	Study plan: N-MIT·HCl: Two Year Storage Stability and Statement, AgroChemex Environmental Ltd., Study number: ENV-20-364, GLP, Unpublished	Yes	Roche	Yes	Yes
Ruckstuhl, D.	2019	5.1a, 3.6-1	2-Methyl-4-isothiazolin-3-one hydrochloride: Five batch analysis of substance identity and composition according to BPR regulation, Intertek (Schweiz) AG, Report No. 18.222, GLP, Unpublished	Yes	Roche	Yes	Yes
Ruckstuhl, D.	2017	5.1b	Validation of a qNMR Method for Content Determination of N-MIT HCl, Intertek (Schweiz) AG, Report No. 17.237-2, Unpublished	Yes	Roche	Yes	Yes
Harrington, P.	2019	5.1c, 3.6-2	Determination of water and chlorine content and UV/VIS spectra in 2-methyl-4-isothiazolin-3-one hydrochloride according to BPR regulation, INTERTEK PHARMACEUTICAL SERVICES MANCHESTER, Report No. 18.222, GLP, Unpublished	Yes	Roche	Yes	Yes
Ruckstuhl, D.	2019	5.1d, 3.6-3	2-Methyl-4-isothiazolin-3-one hydrochloride: Five batch analysis of substance identity and composition according to BPR regulation, Intertek (Schweiz) AG,	Yes	Roche	Yes	Yes

			Report No. 19.225, GLP, Unpublished				
Ruckstuhl, D.	2019	5.1e	2-Methyl-4-isothiazolin-3-one hydrochloride: Five batch analysis of substance identity and composition according to BPR regulation: Determination of the content of chloride, water and the recording of UV-spectrum, Report No. 19082916G404, Intertek (Schweiz) AG, GLP, Unpublished	Yes	Roche	Yes	Yes
Ruckstuhl, D.	2019	5.1f	Preliminary test of 2-methyl-4-isothiazolin-3-one hydrochloride five batch analysis 19.225, Report No. 19.225-A, Intertek (Schweiz) AG, Unpublished	Yes	Roche	Yes	Yes
Reiss, T.	2020	5.1g	Statement on batch numbers for 5-batch analysis, Sigma Aldrich, Unpublished	Yes	Roche	Yes	Yes
Marbo, M.	2005	5.2.1 (LoA)	Validation of CIS analytical methods to determine RH-886 and RH-573 in soil and sediment Samples, Rohm and Haas, Report No. GLP-2005-009, GLP, Unpublished	Yes	Dow (LoA)	Yes	Yes
Kirchherr, M.	2020	5.2.2	2-Methyl-4-isothiazolin-3-one Hydrochloride Validation of an Analytical Method for the Determination in Air, Report No. S19-22203, Eurofins Agroscience Services EcoChem GmbH, GLP, Unpublished	Yes	Roche	Yes	Yes
Krainz, A.	2007	5.2.3a (LoA)	Test method for the determination of 2-methyl-4-isothiazolin-3-one (RH-573) in drinking, surface and sea water, RCC, Report No. A41084, GLP, Unpublished	Yes	Dow (LoA)	Yes	Yes
Krainz, A.	2007	5.2.3b (LoA)	Development and validation of residue analytical methods for the determination of 2-methyl-4-isothiazolin-3-one (RH-573) in drinking, surface and sea water, RCC Ltd, Report No. GLP-2007-019, GLP, Unpublished	Yes	Dow (LoA)	Yes	Yes
Taoudi, M.	2019	5.2.4	Method validation for the determination of 2-methyl-4-isothiazolin-3-one hydrochloride in tissue and body fluid matrices, Battelle UK Ltd., Report No. JC/18/006, GLP, Unpublished	Yes	Roche	Yes	Yes
Moseley, R.	2018	6.0	Expert Statement "Why hydrochloride does not contribute to the biocidal effect of N-MIT·HCl & is not relevant for read-across", ERM, Report No. 0372224-ES1, Unpublished	Yes	Roche	Yes	No
Anonymous	2018	6.5-01	Mode of action - Krebs cycle schematic, ERM, Report No. N/A, Unpublished	No	N/A	Yes	No

Paulus, W.	1993	6.5-02	Microbiocides for the protection of materials. London: Chapman & Hall, 1993: 325 p.14, Published	No	N/A	Yes	No
Brözel, V.S., Cloete, T.E.	1994	6.7.1-01	Resistance of *Pseudomonas aeruginosa* to isothiazolone, Journal of Applied Bacteriology, 76, pp.576-582, Published	No	N/A	Yes	Yes
Périamé, M., Pagès, JM., Davin-Regli, A.	2015	6.7.1-02	*Enterobacter gergoviae* membrane modifications are involved in the adaptive response to preservatives used in cosmetic industry, Journal of Applied Microbiology, Published	No	N/A	Yes	Yes
Evaluation of health hazards							
Anonymous 1	2017	8.1	N-MIT·HCl: An Assessment of *in vitro* Skin Corrosion using EpiDerm™, Covance Laboratories Ltd., Report No. 8366775, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous 2	2002		Repeated Dose 28-Day Oral Toxicity Study of ACTICIDE M 50 in Rats, Report No. 3492, GLP, Unpublished, Summary published in CLH Report for MIT*	Yes	Thor GmbH	No	Yes
Anonymous 3	2002		Repeated Dose 90-Day Oral Toxicity Study of ACTICIDE M 50 in Rats, Report No. 3493 GLP, Unpublished, Summary published in CLH Report for MIT*	Yes	Thor GmbH	No	Yes
Anonymous 4	2000		Investigation of Acticide SR 3267 on Mutagenicity by the Reverse Mutation Assay in Salmonella typhimurium (Ames-test). Report no. 056 TOX 98, GLP, Unpublished, Summary published in CLH Report for MIT*	Yes	Thor GmbH	No	Yes
Anonymous 5	2003	8.3.1-01 (LoA)	Methylisothiazolone: Local lymph node assay, Calvert Laboratories, Report No. 0787XR07.002, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 6	2017	8.3.1-02	Expert opinion on skin sensitisation potential of N-MIT·HCl (2-methyl-4-isothiazolin-3-one hydrochloride), JSC International Limited, Report No. 0372224-TOX1, Unpublished	Yes	Roche	Yes	Yes
Anonymous 7	2018	8.5.1	N-MIT·HCl: Bacterial Reverse Mutation Assay, Covance Laboratories Ltd., Report No. 8366777, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous 8	2000	8.5.2 (LoA)	Mutagenicity test on Kordek 573T: measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells, Covance Laboratories, Report No. 99RC-133, GLP, Unpublished	Yes	Dow Europe GmbH (LoA	Yes	Yes

Anonymous 9	2000	8.5.3 (LoA)	Kordek™ 573T: Test for chemical induction of gene mutation at the HGPRT locus in cultured Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay, Sitek Research Laboratories, Report No. 99RC-265, GLP, Unpublished	Yes	Dow Europe GmbH (LoA	Yes	Yes
Anonymous 10	2018	8.7.1	N-MIT·HCl: Acute Oral Toxicity Study in the Female Rat (Up and Down Method), Covance Laboratories Ltd., Report No. 8373386, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous 11	1995	8.7.2-01 (LoA)	RH-573 Technical: acute inhalation toxicity study in rats, Rohm and Haas Company, Report No. 95R-113, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 12	2001	8.7.2-02 (LoA)	Kordek™ 573F: acute inhalation toxicity study in rats (2001), Rohm and Haas Company, Report No. 01R-100, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 13	2002	8.7.2-03 (LoA)	Kordek™ 573F: acute inhalation toxicity study in rats (2002), Rohm and Haas Company, Report No. 01R-100A, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 14	1999	8.7.3 (LoA)	Kordek™ 573T: acute dermal toxicity study in male and female rats, Rohm and Haas Company, Report No. 99R-061A, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 15	2003	8.8.1-01 (LoA)	Tissue distribution of 14C-RH-573 in the mouse, XenoBiotic Laboratories, Inc, Report No. 03RC-042, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 16	2005	8.8.1-02 (LoA) 8.8.1-03 (LoA)	Metabolism and pharmacokinetics of 14C-RH-573 in the rat, XenoBiotic Laboratories, Report No. 03RC-043, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 17	2005	8.8.1-03 (LoA)	Metabolism of ^{14}C-RH-573 in the biliary cannulated rat, XenoBiotic Laboratories, Report No. RPT01215, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 18	2003	8.8.2-01 (LoA)	*In vitro* percutaneous absorption through rat skin, Rohm and Haas Company, Report No. 00R-066, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 19	2005	8.8.2-02 (LoA)	2-Methyl-4-isothiazolin-3-one (MIT): *in vitro* absorption from water and three formulations through human epidermis, Report No. 04RC-066, Rohm and Haas Company, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 20	2004	8.9.2.1 (LoA)	2-Methyl-4-isothiazolin-3-one: A 13-week dietary toxicity study in dogs, MPI Research, Inc., Report No. 285-069, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 21	2000	8.9.3.1 (LoA)	RH-573 Technical: three month drinking water toxicity study in rats, Rohm and Haas Company	Yes	Dow Europe GmbH	Yes	Yes

			Report No. 99R-135, GLP, Unpublished		(LoA)		
Anonymous 22	1984	8.9.2.2 (LoA)	Kathon™ 886 MMPA Process: thirteen-week inhalation toxicity study in rats, Rohm and Haas Company, Report No. 82R-245, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 23	2000		Acute Dermal Toxicity Study of Acticide SR 3267 in Rat - Limit Test; Report No. 055 TOX 98, GLP, Unpublished, Summary published: CLH Report for MIT*	Yes	Thor GmbH	No	Yes
Anonymous 24	1982	8.9.2.3 (LoA)	Kathon 886 MW: 90-day percutaneous toxicity study in rabbits, Rohm and Haas Company, Report No. 80R-119, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 25	2003a	8.10.1-01 (LoA)	An oral (gavage) developmental toxicity study of 2-methyl-4-isothiazolin-3-one in rats, WIL Research Labs Study, Report No. 01RC-269, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 26	2003b	8.10.1-02 (LoA)	An oral (gavage) developmental toxicity study of 2-methyl-4-isothiazolin-3-one in rabbits, WIL Research Labs, Report No. 02RC-122, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 27	2003c	8.10.2 (LoA)	A two-generation reproductive toxicity study of 2-methyl-4-isothiazolin-3-one administered via drinking water in rats, WIL Research Laboratories, Inc., Report No. 01RC-285, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 28	2007	8.10.2 (LoA)	A two-generation reproductive toxicity study of 2-methyl-4-isothiazolin-3-one administered via drinking water in rats – Protocol 01P-285 Histopathology of the Brain, Research Pathology Services, Inc., Report No. 01RC-285A, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 29	1994	8.11.1-01 (LoA)	Kathon™ biocide: 24-month drinking water chronic/oncogenic study in rats, Rohm and Haas Company, Report No. 90R-149, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Anonymous 30	1983	8.11.2-01 (LoA)	30-month dermal carcinogenesis study in male mice, Rohm and Haas Company, Report No. 81R-288, GLP, Unpublished	Yes	Dow Europe GmbH (LoA)	Yes	Yes
Lundov, M.D. et *al.*	2011	8.12-01	Methylisothiazolinone contact allergy: a review, British Journal of Dermatology, Volume 165, Issue 6, pp. 1178 – 1182, Published	N/A	N/A	Yes	Yes
Mose, A.P. et *al.*	2012	8.12-03	Occupational contact dermatitis in painters – an analysis of patch test data from the Danish Contact	N/A	N/A	Yes	Yes

			Dermatitis Group, Contact Dermatitis, 67, 293–297, Published				
Anonymous 31	2000		Acute Inhalation Toxicity Study of Test Item Acticide SR 3267 in Rats; Report No. 99/419-004P, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Thor GmbH	No	Yes
Anonymous 32	1999		Kordek™ 573T: Salmonella typhimurium gene mutation assay Rohm and Haas Company, Rohm and Haas Report N° 99R-062; Unpublished, Summary published: CLH Report for MIT*	No	Rohm and Haas	No	Yes
Anonymous 33	2002		In vitro Mammalian Chromosome Aberration Test of ACTICIDE M 50 with Human Lymphocytes;Report No. 3754, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Thor GmbH	No	Yes
Anonymous 34	2000		Mutagenic Evaluation of Test Item Acticide SR 3267 in CHO/HPRT Assay; Report No. 99/419-015M, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Thor GmbH	No	Yes
Anonymous 35	2000		Kordek™ 573T: micronucleus assay in CD-1 mouse bone marrow cells, Rohm and Haas Company, Rohm and Haas Report N° 99R-132; Unpublished, Summary published: CLH Report for MIT*	No	Rohm and Haas	No	Yes
Anonymous 36	2000		Mutagenic Effect of Test Item ACTICIDE SR 3267 by Micronucleus Test; Report No. 99/419-013M, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Thor GmbH	No	Yes
Anonymous 37	2003		2-Methyl-4-isothiazolin-3-one (RH-573): In Vivo/In Vitro unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with a dose range finding assay, Covance Laboratories Study N° 25074-0-494 OECD, Rohm and Haas Report N° 03RC044; Unpublished, Summary published: CLH Report for MIT*	No	Rohm and Haas	No	Yes
Anonymous 38	1994		Study to Evaluate the Potential of ACTICIDE 14 to Induce Unscheduled DNA Synthesis in Rat Liver using an in vivo/in vitro Procedure; Report No. 1154/24, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Thor GmbH	No	Yes

Anonymous 39	2003	8.10.1-01	An oral (gavage) developmental toxicity study of 2-methyl-4-isothiazolin-3-one in rats, WIL Research Labs, Report No. WIL-91012, Rohm and Haas Report N° 02RC-122, GLP, Unpublished, Summary published: CLH Report for MIT*	No	Rohm and Haas	No	Yes
Anonymous 40	2003		A Two-Generation reproductive development toxicity study of 2-Methyl-4-isothiazolin-3-one administered via drinking water in rats, Report No. WIL-91005, GLP, Unpublished, Summary published in CLH Report for MIT*	Yes	Thor GmbH	No	Yes
Evaluation of environmental hazards							
Anonymous	2017a	9.1.2.1-01	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Acute Toxicity to *Daphnia magna* in a Static 48-hour Immobilisation Test, ibacon GmbH, Report No. 123831220, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous	1997	9.1.3 (LoA)	Toxicity of RH-573 technical to the freshwater alga, *Selenastrum capricornutum*, TR Wilbury Laboratories Study No: 1036-RH, Rohm and Haas Report No: 95RC-164, March 25, 1997, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2017b	9.1.3.1-01	2-Methyl-4-Isothiazolin-3-One Hydrochloride: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test, ibacon GmbH, Report No. 123831210, GLP, Unpublished	Yes	Roche	Yes	Yes
Anonymous	1996	9.1.5 (LoA)	Activated sludge respiration inhibition test with RH-573 technical, TR Wilbury Study N° 1037-RH, Rohm and Haas Report No: 95RC-0165, September 26, 1996, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2005	9.1.6.1 (LoA)	Early life-stage toxicity of 2-methyl-4-isothiazolin-3-one to the rainbow trout, *Oncorhynchus mykiss*, under flow-through conditions. ABC Laboratories Study No: 48835. Rohm and Haas Report N° 04RC-023 (March 11, 2005), Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2004	9.1.6.2 (LoA)	2-Methyl-4-isothiazolin-3-one: Chronic toxicity test with the water flea, *Daphnia magna*, conducted under flow-through conditions. ABC Laboratories Study No: 48836, Rohm and Haas Rep Report No: 04RC-0024, November 8, 2004, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	1992	10.1.1.1.a	Hydrolysis of ^{14}C RH-573 at pH 5, 7, and 9; Pharmacology and	Yes	Rohm and	Yes	-

		(LoA)	Toxicology Research Laboratory-West, Richmond, CA USA, PTRL Report N° 223W-1 Rohm and Haas Company, Technical Report No: 34-92-63, November 6, 1992, Unpublished		Haas (LoA)		
Anonymous	1995	10.1.1.1.b (LoA)	Sunlight Photodegradation of ^{14}C RH-573 (the Minor Component of RH-886) in a Buffered Aqueous Solution at pH 7; PTRL West, Inc. Richmond, CA, USA, PTRL Project N° 224W, Rohm and Haas Technical Report No: 34-94-78, May 4, 1995, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2017c	10.1.1.2	Methyl isothiazolinone hydrochloric acid (N-MIT·HCl): Assessment of Ready Biodegradability; CO_2 Evolution Test, Envigo Research Limited, Report No. SB47QQ, Unpublished	Yes	Roche	Yes	Yes
Anonymous	2003a	10.1.1.2/01 (LoA)	Ready Biodegradation of N-methyl Malonamic Acid in a CO_2 Evolution (Modified Sturm) Test; RCC Ltd, CH-4452 Itingen, Switzerland, RCC Study No: 843966, Rohm and Haas Report No: GLP-2002-081, April 22, 2003, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2003b	10.1.1.2/02 (LoA)	Ready Biodegradation of N-methyl Acetamide in a CO2 Evolution (Modified Sturm) Test; RCC Ltd, CH-4452 Itingen, Switzerland, RCC Study No: 843967, Rohm and Haas Report No: GLP-2003-031, November 5, 2003, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2003c	10.1.1.2/03 (LoA)	Ready Biodegradation of Malonamic Acid in a CO_2 Evolution (Modified Sturm) Test; RCC Ltd, CH-4452 Itingen, Switzerland, RCC Study No: 843968, Rohm and Haas Report No: GLP-2003-032, November 5, 2003, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2006	10.1.2, 10.2.4 (LoA)	RH-573: Adsorption and Desorption to Soil; Brixham Environmental Laboratories, Brixham, Devon, UK. Brixham Report No: BL8308/B, Rohm and Haas Technical Report No: 06-058, August 29, 2006, Unpublished	Yes	Rohm and Haas (LoA)	Yes	-
Anonymous	2018	10.3.1	2-Methyl-4-Isothiazolin-3-One Hydrochloride, CAS Number 26172-54-3: Estimation of Atmospheric Oxidation Rate, ERM, Report No. 0372224-AOR1, Unpublished	Yes	Roche	Yes	Yes
Anonymous	2018	13-1	N-MIT·HCl Read-across to MIT – WG Proposal, ERM, Report No. 0372224-WG2, Unpublished	Yes	Roche	Yes	Yes
Anonymous	2021	13-1a	Justification for the use of read-across approaches for hazard prediction of N-MIT·HCl, ERM,	Yes	Roche	Yes	Yes

			Report No. 0372224-TOX1 Amendment 1, Unpublished				
Quérou, R.	2018	13-2	Letter of Access to the European Chemicals Agency, Dow Europe GmbH, Report No. N/A, Unpublished	N/A	Roche	Yes	Yes
Quérou, R.	2019	13-2a	Letter of Access to the European Chemicals Agency, Dow Europe GmbH, Report No. N/A, Unpublished	N/A	Roche	Yes	Yes
Anonymous	2019	13-3	Safety Data Sheet, Roche Diagnostics Deutschland GmbH, Report No. N/A, Unpublished	No	Roche	Yes	Yes

Roche = Roche Diagnostics GmbH

* CLH Report For [2-methylisothiazol-3(2H)-one (MIT)], 2015, https://echa.europa.eu/documents/10162/0d4c2335-6009-4e65-9278-c7f10a3a2dad.

APPENDIX VII: STUDY SUMMARIES

Study summaries for the assessment are extracted from IUCLID and are available in Annex I.

APPENDIX VIII: READ ACROSS BETWEEN N-MIT·HCL AND N-MIT

A justification for the read across from already submitted and approved N-MIT (free base) to the new active substance N-MIT·HCl has been submitted by using the ECHA Read-Across Assessment Framework (RAAF, 2017a). Document is available in Annex II.