Committee for Risk Assessment

RAC

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

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

EC Number: -

CAS Number: 2527-66-4

CLH-O-0000001412-86-209/F

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

Adopted

8 June 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 2-Methyl-1,2-benzisothiazol-3(2H)-one; [MBIT]

EC Number: -

CAS Number: 2527-66-4

Index Number: -

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Version number: 3 **Date: 08.05.2017**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	2-Methyl-1,2-benzisothiazol-3(2H)-one (IUPAC name) N-methyl-1,2-benzisothiazol-3(2H)-one; methylbenzisothiazolone; ***MBIT*** (the abbreviated common name)
EC number:	-
CAS number:	2527-66-4
Annex VI Index number:	-
Degree of purity:	≥99.7%
Impurities:	***Confidential***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Currently not in Annex VI
Current proposal for consideration by RAC	**Classification:** Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 3, H331 Skin Corr. 1B, H314 Eye Dam. 1, H318

	Skin Sens. 1A, H317 Aquatic Acute 1, H400 Aquatic Chronic 2, H411 Acute M factor: M=1 **Labelling** Pictograms: GHS05, GHS06, GHS09 Signal word: Dgr Hazard statements: H301, H311, H331, H314, H317**,** H410* *Article 27 of CLP states that if a substance or mixture is classified within several hazard classes or differentiations of a hazard class, all hazard statements resulting from the classification shall appear on the label, unless there is evident duplication or redundancy. This means that where a substance or a mixture is classified both in acute and long-term hazard categories, the hazard statement required to appear on the label shall for this hazard classification be H410
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	**Classification:** Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 3, H331 Skin Corr. 1B, H314 Eye Dam. 1, H318 Skin Sens. 1A, H317 Aquatic Acute 1, H400 Aquatic Chronic 2, H411 Acute M factor: M=1 **Labelling** Pictograms: GHS05, GHS06, GHS09 Signal word: Dgr Hazard statements: H301, H311, H331, H314, H317, H410* *Article 27 of CLP states that if a substance or mixture is classified within several hazard classes or differentiations

	of a hazard class, all hazard statements resulting from the classification shall appear on the label, unless there is evident duplication or redundancy. This means that where a substance or a mixture is classified both in acute and long-term hazard categories, the hazard statement required to appear on the label shall for this hazard classification be H410.

1.3 Proposed harmonised classification and labelling based on CLP Regulation criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for

					classification

2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 3, H301	Not applicable	Not classified	
	Acute toxicity - dermal	Acute Tox. 3, H311	Not applicable	Not classified	
	Acute toxicity - inhalation	Acute Tox. 3, H331	Not applicable	Not classified	
3.2.	Skin corrosion / irritation	Skin Corr. 1B, H314	Not applicable	Not classified	
3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318	Not applicable	Not classified	
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Skin Sens. 1A, H317	Not applicable	Not classified	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	No applicable
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 2, H411	M= 1	Not classified	

5.1.	Hazardous to the ozone layer	Not classified		Not classified	Data conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictograms: GHS05, GHS06, GHS09

Signal word: Dgr

Hazard statements:

H301: Toxic if swallowed.

H311: Toxic in contact with skin.

H331: Toxic if inhaled.

H314: Causes severe skin burns and eye damage.

H317: May cause an allergic skin reaction.

H410 : Very toxic to aquatic life with long lasting effects.

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

None. No notes are proposed.

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

A harmonised classification for 2-Methyl-1,2-benzisothiazol-3(2H)-one (MBIT) is not available in Annex VI of the Regulation (EC) No 1272/2008.

1.5 Short summary of the scientific justification for the CLH proposal

No classification is warranted for physico-chemical hazards.

With an oral LD_{50} of 175 mg/kg bw, MBIT warrants the classification Acute Tox. 3; H301.
With an dermal LD_{50} of > 200 mg/kg bw, MBIT warrants the classification Acute Tox. 3; H311.
With an inhalation LC_{50} of > 0.53 mg/L, MBIT warrants the classification Acute Tox. 3; H331.

Under the conditions of skin corrosion/irritation study, MBIT was considered to be a skin corrosive and should be classified, according to CLP Regulation, in subcategory 1B – responses occur after 1 hour exposure and observations up to 14 days.
According to CLP Regulation skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). Taking into account the above mentioned information Dossier Submitter recommends classification, according to CLP, of MBIT as corrosive to the eyes of rabbits – Eye Dam. 1, H318.

Based on the following information:
- the results of two Local lymph node assay (LLNA) studies (first study: the Stimulation Index was above 3 at MBIT concentration 1.04%, what fulfils the criteria for Skin Sens. 1A, EC3 value ≤ 2%, second study: the Stimulation Index was above 3 at MBIT concentration 0.69%, what fulfils the criteria for Skin Sens. 1A, EC3 value ≤ 2%).
- the result of Buehler test (when MBIT was tested by using Buehler method at 0.18% (1800 ppm (229 µg/cm2)), a minimal incidence (20%) of delayed contact hypersensitivity in guinea pigs was observed, what fulfils the criteria for Skin Sens. 1A),
- one human repeated insult patch test (HRIPT) study (additional supporting information)
it can be concluded that MBIT should be classified, according to CLP Regulation, as skin sensitizer (Skin Sens. 1A, H317 – May cause an allergic reaction).

In a ready biodegradation studies, MBIT was found not to be ready biodegradable. Nevertheless, biological half-lives in the environment are very short:
- the half-life of MBIT in ready biodegradability test is estimated to be less than 2.2 days,
- MBIT biodegrades very quickly in the fresh surface water studied. The half-live is 0.05 days at 12°C,

- the half-life of MBIT in the simulated Sewage Treatment Plant (STP) system was 0.32 hour.

Metabolism of degradation involved cleavage of the isothiazolone ring.

Simulation tests show rapid primary biodegradation of MBIT in the environment. According to the Guidance on the Application of CLP criteria (Version 4.1 – June 2015) data on primary degradation can only be used to show rapid degradation of substance where it is demonstrated that the degradation products shall not be classified as hazardous to the environment, i.e. that they do not fulfil the classification criteria. Main metabolites identified during degradation of MBIT are:

- N-Methyl-2-(Methylthio)Benzamide,
- 2-(methylcarbamoyl)- benzene sulfonic acid,
- 2-carbamoyl- benzene sulfonic acid.

Taking into account the results of environmental tests performed on metabolites of MBIT it can be concluded that these metabolites should not be classified, according to CLP Regulation, as hazardous to the environment.

Taking into account the available data:

- simulation tests show rapid primary biodegradation of MBIT in the environment,
- the degradation products N-Methyl-2-(Methylthio)Benzamide, 2-(methylcarbamoyl)-benzene sulfonic acid and 2-carbamoyl-benzene sulfonic acid are not classified as hazardous to the environment, it can be concluded that MBIT is rapidly degradable for the purposes of aquatic hazard classification.

The lowest available $L(E)C_{50}$ value relevant for classification of MBIT is the 48h ErC_{50} of 0.24 mg a.i./L obtained for the *Pseudokirchneriella subcapitata* and 96h LC_{50} of 0.24 mg a.i./L for *Oncorhynchus mykiss*. Based on these lowest $L(E)C_{50}$ values MBIT fulfils the criteria $L(C)E_{50} \leq 1$ mg/L for classification as Acute Aquatic Category 1, H400 (Very toxic to aquatic life) with M-factor of 1 due to 48h ErC_{50} and 96h LC_{50} is in the range $0.1 < L(E)C50 \leq 1.0$ mg/L.

The lowest $NOEC/EC_{10}$ is the 48 hours NOEC of 0.012 mg a.i./L obtained for freshwater alga species *Pseudokirchneriella subcapitata*. Available NOEC values for fish and Daphnia are higher. The lowest endpoint for MBIT for algae fulfils the criteria 0.01 mg/l > NOEC/ECx ≤ 0.1 mg/L (for substance readily biodegradable – Table 4.1.0 b) (ii)) for classification as Aquatic Chronic 2, H411 (Toxic to the aquatic organisms with long lasting effects).

In accordance with the provisions of CLP Regulation MBIT should be classified as hazardous to the environment:

- Aquatic Acute 1, H400; M=1
- Aquatic Chronic 2, H411

The majority of the tests have been conducted with the pure active substance (purity ≥99.7% w/w), only the acute inhalation toxicity study was performed on formulated product (mixture containing 24% of MBIT).

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

Not included in Annex VI to CLP Regulation.

1.7 Current self-classification and labelling

2-Methyl-1,2-benzisothiazol-3(2H)-one (MBIT) (CAS No 2527-66-4) is classified by notifiers in C&L Inventory as below:

Summary of Classification and Labelling

Notified classification and labelling

General Information

EC Number	EC Name	CAS Number
		2527-66-4

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Classification affected by Impurities / Additives	Additional Notified Information	Number of Notifiers	Joint Entries	Zobacz
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)							
Acute Tox. 3	H301	H301		GHS06 GHS09 GHS05 Dgr				State/Form IUPAC Names	47		
Acute Tox. 4	H312	H312									
Skin Irrit. 2	H315	H315									
Skin Sens. 1A	H317	H317									
Eye Dam. 1	H318	H318									
Acute Tox. 3	H331										
Aquatic Acute 1	H400	H400									
		H335									

Number of Aggregated Notifications: 1

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

MBIT is an active substance in the meaning of Directive 98/8/EC (repealed by Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products) and therefore subject to harmonized classification and labelling (Regulation EC 1272/2008 article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

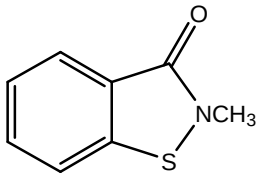
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	MBIT is a new biocidal active substance. It is not listed in EINECS chemical.
EC name:	-
CAS number (EC inventory):	-
CAS number:	2527-66-4
CAS name:	1,2-Benzisothiazol-3-(2H)-one, 2methyl-
IUPAC name:	2-methyl-1,2-benzisothiazol-3(2H)-one
Common name	N-methyl-1,2-benzisothiazol-3(2H)-one
Manufacturer´s development code number(s)	methylbenzisothiazolone MBIT
CLP Annex VI Index number:	-
ISO name	There is no ISO name. The abbreviated common name is MBIT (this is the name used in this dossier).
Molecular formula:	C_8H_7NOS
Molecular weight range:	165.215

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
2-methyl-1,2-benzisothiazol-3(2H)-one		> 997 g/kg	

Current Annex VI entry: not included in Annex VI

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
None of the impurities is considered relevant for classification purposes. The information about impurities are included in IUCLID file.			

Current Annex VI entry: not included in Annex VI

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
none				

Current Annex VI entry: not included in Annex VI

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Crystalline solid	Bates, ML (2009c);	Purity: 99.7% (specified on certificate of analysis)
Melting/freezing point	53.3 °C	Bates, ML (2009a);	measured; method EC92/69/EEC A1 and OECD Guideline 102
Boiling point	324.6 °C	Bates, ML (2009a)	measured; method EC92/69/EEC A2 and OECD Guideline 103
Relative density	D^{20}_4 = 1.4527	Bates, ML (2009b)	measured; method A3 and OECD Guideline 109
Vapour pressure	23.4 mPa at 20°C. 42.5 mPa at 25°C.	Bates, ML (2009a)	measured; method EC92/69/EEC A4 and OECD Guideline 104
Surface tension	Temperature: 19.8 °C. Concentration: 1.0 g/L Result: 60.8 mN/m	Bates, ML (2007)	measured; method EC92/69/EEC A5 and OECD Guideline 115
Water solubility	Nominal pH 3.4: 14.63 g/L at 20.1 °C Nominal pH (5.1): 10.35 g/L at 7.8 °C 15.08 g/L at 20.1 °C 38.57 g/L at 35.2 °C Nominal pH 8.0: 15.97 g/L at 20.1 °C	Bates, ML (2009a)	measured; method EC92/69/EEC A6 and OECD Guideline 105
Partition coefficient n-octanol/water	pH neutral: log K_{ow} = 1.42, at 10 °C log K_{ow} = 1.40 at 20 °C log K_{ow} = 1.39 at 30 °C Buffered at pH=3.4: log K_{ow} = 1.52 at 20 °C Buffered at pH=8.0: log K_{ow} = 1.41 at 20 °C	Bates, ML (2009a)	measured; method EC92/69/EEC A8 and OECD Guideline 117

Flash point	The test substance is a solid at ambient temperature with peak of melting point above 50°C. Flammability has been adequately addressed to EEC data guidelines A10 (Flammability: Solids) and A16: Relative Self Ignition Temperature.	Bates, ML (2009c)	Not applicable
Flammability	The test substance did not ignite, under the conditions of the test, but melted to a yellow liquid, which turned solid when the flame was removed. It is therefore classified as not highly flammable.	Bates, ML (2009c)	measured; method EC92/69/EEC A10
Explosive properties	1. There were no exothermic events in the DSC thermogram. 2. There are no functional groups of (explosivity) concern in the molecular structure. 3. The oxygen balance (-179.16%) is within the region of concern, but close to the limit value of -200%. Overall, the combination of these factors, and the clear lack of adverse thermal properties indicate that the test substance is unlikely to possess explosive properties.	Bates, ML (2009c)	measured; method EC92/69/EEC A14
Self-ignition temperature	No relative self-ignition was observed up to 400°C. After heating, physical state of the test substance had changed from a cream colored solid (prior test) to a red-brown crispy solid	Bates, ML (2009c)	measured; method EC92/69/EEC A16

	residue, indicating that the test substance had decomposed.		
Oxidising properties	1. There were no exothermic events in the DSC thermogram. 2. Structurally, There are no functional groups of (oxidising) concern. 3. The oxygen balance (-198.53%) is just within the region of concern where a potential for oxidation exists but very close to the limit value. Overall, the combination of these factors, and the clear lack of adverse thermal properties indicate that the test substance is unlikely to possess oxidising properties.	Bates, ML (2009c);	expert assessment; method EC92/69/EEC A17/A21
Granulometry			
Stability in organic solvents and identity of relevant degradation products	Active substance as manufactured does not contain any organic solvents.	(N/A)	-
Dissociation constant	The derived dissociation constant for MBIT was: -2.0. However, this should not be taken as a definitive value as it is outside the range of the instrument, and was derived by extrapolation. This derived dissociation constant is outside the range of normal environmental interest.	Bates, ML (2009a);	measured; method OECD Guideline 112
Viscosity	Not applicable (N/A)		Test substance is a solid.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for classification and labelling.

2.2 Identified uses

MBIT is widely used preservative (Product type 6 (In-can preservatives), 13 (Metal working fluid preservatives) according to Annex V of Regulation (EU) No. 528/2012).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
See Table 8			

3.1 Physico-chemical hazards

3.1.1 Summary of physico-chemical properties

Based on the results of test data 2-methyl-1,2-benzisothiazol-3(2H)-one (MBIT) is not explosive, oxidizing, flammable. The MBIT can be considered as thermally stable at room temperature. No flash point was determined as the substance is a solid and does not have a melting point below 40°C. The summaries included in this proposal are copied from CAR. For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarized in Table 8.

3.1.2 Comparison with criteria

Not relevant

3.1.3 Conclusions on classification and labelling

No classification is required.

RAC evaluation of physical hazards
Summary of the Dossier Submitter's proposal The Dossier submitter (DS) did not propose classification for physical hazards. All of the relevant physico-chemical hazards were assessed according to test methods described in Part I of the UN Recommendations on the Transport of Dangerous Goods – Model Regulations (UN RTDG). Based on these results, the DS concluded that MBIT is not flammable, explosive, or oxidising. It can be considered as thermally stable at room temperature. No flash point was determined as the substance is a solid and does not have a melting point below 40 °C. Comments received during public consultation No comments were received regarding physical hazards. Assessment and comparison with the classification criteria There were no indications of physical hazards based on the test results available. MBIT is not explosive, oxidizing, flammable. Therefore, RAC agrees with the DS that classification is not required for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 10: Summary of toxicokinetics (absorption, metabolism, distribution and elimination): non human information.

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>OECD Guideline 417

Species: rat

Strain: Sprague-Dawley

Sex: males and females

oral: gavage

[^{14}C]-MBIT was formulated with 0.5% methylcellulose in water. 0.5% methylcellulose was used as the dosing vehicle.

Intact and jugular vein cannulated (JVC) male and female Sprague Dawley rats were dosed orally with either 10 mg/kg or 100 mg/kg in this study. One group of rats was dosed with 5 consecutive doses of 10 mg/kg/day.
One additional rat per gender was not dosed, and these served as controls to generate blank matrices.</td>
<td>
<table>
<tr><th>Group</th><th>No. of rats/sex</th><th>Dose (mg/kg)</th><th>Radio-activity µCi/kg</th><th>Dose Volume (mL/kg)</th><th>Sample Collection[a]</th></tr>
<tr><td>1</td><td>4</td><td>10</td><td>~100</td><td>~10</td><td>Urine, feces, tissue, carcasses, blood, plasma</td></tr>
<tr><td>2</td><td>3 JVC</td><td>10</td><td>~100</td><td>~10</td><td>Blood/plasma</td></tr>
<tr><td>3</td><td>4</td><td>100</td><td>~100</td><td>~10</td><td>Urine, feces, tissues, carcasses, blood, plasma</td></tr>
<tr><td>4</td><td>3 JVC</td><td>100</td><td>~100</td><td>~10</td><td>Blood/plasma</td></tr>
<tr><td>5</td><td>3</td><td>10</td><td>~100</td><td>~10</td><td>Blood, plasma, tissues</td></tr>
<tr><td>6</td><td>3</td><td>10/day for 5 days</td><td>~100/ day</td><td>~10</td><td>Urine, feces, tissues, carcasses, blood, plasma</td></tr>
<tr><td>7[b]</td><td>1</td><td>NA</td><td>NA</td><td>NA</td><td>Urine, feces, tissues, blood/plasma</td></tr>
</table>

Following a low dose of 10 mg/kg of [^{14}C]-2-Methyl-1,2-benzisothiazolin-3-one), animals appeared normal throughout the study and demonstrated no adverse effects from the test substance administration. However, after a high dose (100 mg/kg), animals appeared lethargic. Male animals recovered, but female animals were more affected by the compound; Animal G4-06-F was found dead in the cage at the afternoon observation post 24-hr sample collection. Blood was also difficult to collect from the high dose group, resulting in some time points with no sample.

Following a single oral low dose of [^{14}C]-MBIT to rats (Group 1, 10 mg/kg), almost all of the dose was recovered in urine and cage rinse (97.08% for males and 99.00% for females) and a very small amount was recovered in faeces (4.03% for males and 5.22% for females). Following a single oral high dose of [^{14}C]-MBIT to rats (Group 3, 100 mg/kg), again, most of the dose was recovered in urine and cage rinse (96.63% for males and 93.41% for females) and a very small amount was found in faeces (3.85% for males and 3.88% for females). Following a single oral low multiple dose of [^{14}C]-MBIT to rats (Group 6, daily for 5 consecutive days at 10 mg/kg/day), 94.69% for males and 93.01% for females was recovered in urine (including cage rinse) and 6.28% for males and 6.35% for females was recovered in faeces. Most of the dosed radioactivity was recovered
</td>
<td>1 (reliable without restriction)

key study

experimental result

Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT)

CAS No: 2527-66-4.</td>
<td>Wu D. and Desai M. (2009)</td>
</tr>
</table>

	within 24 hr post-dose for all groups. Total mean recoveries from all mass balance groups were greater than 97%. There was no gender difference in the excretion pattern. After oral administration, [^{14}C]-MBIT derived radioactivity was rapidly excreted in urine and faeces. Essentially all administered radioactivity was recovered within 24 hr. Urine (including cage rinse) was the major route of excretion (>93%) and a very small amount was found in faeces (3.9% to 6.3%). No gender difference was observed.		

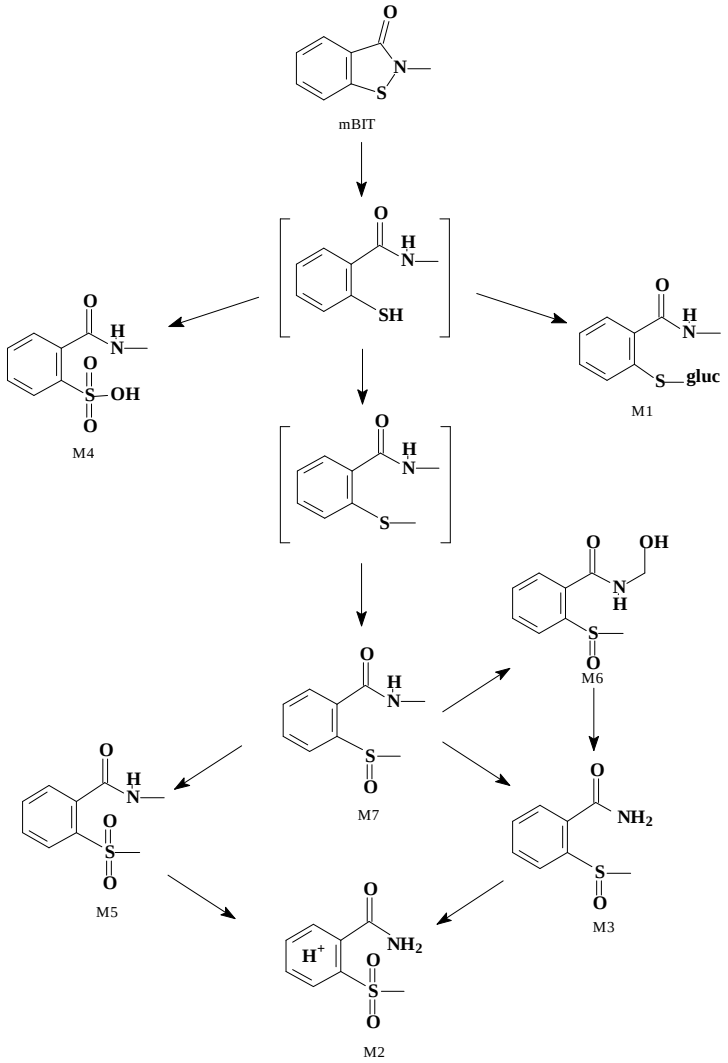
4.1.2 Human information

No other relevant information is available.

4.1.3 Summary and discussion on toxicokinetics

Based on the study conducted in compliance with OECD Guideline 417 it can be concluded that [^{14}C]-MBIT, ([^{14}C]-2-Methyl-1,2-benzisothiazolin-3-one), was rapidly absorbed, extensively metabolized following a single or multiple doses to the rat and rapidly excreted, predominately in the urine. Unchanged MBIT was not found in urine or feces. The metabolite profiles of male and female rat urine from the multiple oral dose group (Group 6) were similar to those of the single dose group. Overall, the findings indicate that MBIT does not bioaccumulate in rat tissues.

The metabolism of MBIT involves thiazolin ring-opening (between sulfur and nitrogen atoms), followed by glucuronyl (M1) or methyl conjugations. Further oxidation of the methyl thiol, N-demethylation, and hydroxylation resulted in the other metabolites of MBIT. The proposed MBIT metabolic pathways are shown below.



4.2 Acute toxicity

The acute toxicity of MBIT has been investigated by the oral (rat) and dermal (rat) route.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

For the acute toxicity by the oral administration route one study was identified as key study and fully summarised.

Table 11: Overview of experimental data on acute toxicity by the oral route.

Method	Results	Remarks	Reference
Test animals Species: rat Strain: Wistar albino Sex: Females OECD Guideline for Testing of Chemicals No. 425 with deviations Environmental conditions Temperature: 14 – 26°C Humidity: 0 – 70%	LD_{50} = 175 mg/kg in females (95% confidence interval 54 to 608 mg/kg) (The acute oral LD_{50} and 95% confidence limits were calculated using AOT425 Statistical program provided by the US EPA).	2 (reliable with restrictions) Key study Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Cerven D.R. (2009a)

The study was conducted in compliance with OECD Guideline 425. The deaths occurred on day 1 with predeath physical signs of lethargy, piloerection, ataxia, prostration, flaccid muscle tone, negative righting reflex, few faeces, tremors, wetness of the nose/mouth area, and laboured breathing. Necropsy results of these rats revealed abnormalities of the thymus, kidneys, liver and gastrointestinal tract.

Under the conditions of this study, MBIT is considered to be classified for acute oral toxicity in category 3 (Acute Tox. 3, H301). Acute oral LD_{50} in female rats = 175 mg/kg body weight (95% confidence limits: 54 to 608 mg/kg bw). Based on this value the proposed Acute Toxicity Estimates (ATE) value, which is used for classification of mixture containing MBIT, is 175 mg/kg body weight.

The results of the acute oral toxicity test are summarized in Table 12.

Table 12: Table for Acute Oral Toxicity rats.

Dose [unit] mg/kg bw	Number of dead / number of investigated	Time of death (range)
55	0/1	Not applicable
175	1/3	Day 1
550	2/2	Day 1
2000	1/1	Day 1
LD_{50} value	Acute oral LD_{50}, rat = 175 mg/kg bw (95% confidence limits 54 to 608 mg/kg/bw)	

4.2.1.2 Acute toxicity: inhalation

Due to the physical chemical properties of the technical material, an acute inhalation study could not be conducted on MBIT. However, when the technical is formulated into an end-use formulation a study could be conducted (an acute inhalation study was

conducted, for the US EPA, on commercial product - a formulation of 24% MBIT in 96% propylene glycol 400. Male and female rats were exposed to one dose (2.22 mg/L) of these commercial product - aerosol - for 4 hours, nose-only. Rats were observed for 14 days post treatment).

Table 13: Overview of experimental data on acute toxicity by the inhalation route.

Method	Results	Remarks	Reference
Test animals Species: rat Strain: Sprague-Dawley Sex: Male and Females Number of animals per group: 5/sex/group Type of exposure: Nose only OECD 403 and US EPA 870.1300 GLP: YES Deviations: No	>2.22 mg/L of air, product (mixture containing 24% MBIT) >0.53 mg a.i. (MBIT)/L of air	1 (reliable without restrictions) Key study Experimental result Test material: a formulation of 24 % MBIT in 96% propylene glycol 400	Younger C. (2009)

A formulation of 24% MBIT was evaluated for its acute inhalation toxicity potential through a nose-only exposure to albino rats. Five males and five females were exposed for four hours to an aerosol generated from the undiluted liquid test formulation at a level of 2.22 mg/L. There was no mortality during the study. Clinical signs included activity decrease, piloerection and respiratory chirps, which were no longer evident by Day 8. Body weights were somewhat affected by exposure, four animals lost or failed to gain weight during the first week. The gross necropsy revealed no observable abnormalities. Results:
Acute 4 hour aerosol Inhalation, rat LC_{50}: > 2.22 mg/L product (a formulation of 24% MBIT)
Acute 4 hour aerosol Inhalation, rat LC_{50}: > 0.53 mg a.i. (MBIT)/L
Because there is no exact value of LC_{50} for inhalation route it is proposed to derived the acute toxicity estimate value for inhalation route from Table 3.1.2 of CLP Regulation that relates to a classification category. Based on the Table 3.1.2 of CLP Regulation ATE value for substances classified for acute inhalation toxicity (mist) in category 3 is equal to 0.5 mg/l.

4.2.1.3 Acute toxicity: dermal

For the acute toxicity by the dermal administration route two studies were performed and fully summarised.

Table 14: Overview of experimental data on acute toxicity by the dermal route.

Method	Results	Remarks	Reference
Test animals Species: rat Strain: Wistar albino	>200 LD_{50} <2000 mg MBIT/kg bw	1 (reliable) Key study Experimental result	Cerven D.R. (2009b)

Sex: males and females OECD Guideline for Testing of Chemicals No. 402		Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	
Test animals Species: rat Strain: Fisher 344 Sex: males and females OECD Guideline for Testing of Chemicals No. 402	LD_{50} >5000 mg mBIT/kg bw	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 98.34% a.i.	Durando J. (2012)

The first study (Cerven D.R. (2009b)) was conducted in compliance with OECD Guideline 402. MBIT was applied to the shaved intact skin of male and female rats for 24 hours, occluded. Rats were observed for 14 days post treatment.

All ten rats died within one day of the 5000 mg/kg dermal application. Under the conditions of this study, MBIT was considered to be >200 $LD_{50} \leq$ 2000 mg/kg, (Acute Tox. 3, H311). Three rats died by day 1 of the 2000 mg/kg dermal application. Body weight changes of the survivors were normal. Instances of erythema, edema, eschar and flaking skin were noted on the 2000 mg/kg treated areas of skin. Necropsy results of the 2000 mg/kg survivors revealed treated skin abnormalities.

Since two of the five female rats survived the 2000 mg/kg dermal application, this indicated that the dermal LD_{50} was slightly below this dosage. The study director judged from these findings that 2-Methyl-1,2-benzisothiazolin-3-one technical would be classified in EPA Category II (that is, the dermal LD_{50} is greater than 200 mg/kg but less than 2000 mg/kg). Therefore, in the interest of conserving animals, particularly since the test material is a strong irritant, no further dosing was conducted in this study.

The dermal LD_{50} of 2-methyl-1,2-benzisothiazol-3(2H)-one technical is less than 2000 mg/kg of body weight in rats (but judged greater than 200 mg/kg). MBIT should be classified according to CLP Regulation for acute dermal toxicity in category 3 (200 < $LD_{50} \leq$ 1000 mg/kg). Because there is no exact value of LD_{50} for dermal route it is proposed to derived the acute toxicity estimate value for dermal route from Table 3.1.2 of CLP Regulation that relates to a classification category. Based on the Table 3.1.2 of CLP Regulation ATE value for substances classified for acute dermal toxicity in category 3 is equal to 300 mg/kg body weight.

Table 15: Table for Acute Dermal LD_{50} Toxicity.

Dose [unit] mg/kg	Number of dead / number of investigated	Time of death (range)	Observations
0	Not applicable		
2000	3/5 females	3 died by day 1	Clinical observations: Wetness of anogenital area and chromodacryorrhea (excretion of red tears). Necropsy: abnormalities of treated skin, pancreas, thymus, and gastrointestinal tract.
5000	5/5 males and 5/5 females	All died within one day	Clinical observations: lethargy, ataxia, and tremors noted in one rat. Necropsy: abnormalities of treated skin, red areas on thymus, red areas on pancreas, yellow staining of the fatty tissue in the peritoneal cavity posterior to the kidney and intestinal abnormalities.
LD_{50} value	Acute Dermal >200 LD_{50} <2000 mg/kg		

The second study (Durando J. (2012)) was conducted in compliance with OECD Guideline 402 and US EPA OPPTS 870.1200. There were no guideline deviations. MBIT was applied to the shaved intact skin of male and female rats for 24 hours, occluded. Rats were observed for 14 days post treatment.

2000 mg/kg bw/day: All animals survived exposure to the test substance and appeared active and healthy during the study. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior. Although all animals lost body weight by Day 1, all animals gained body weight by the end of the 14-day observation period, except for one female, which returned to its initial body weight. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

5000 mg/kg bw/day: All animals survived exposure to the test substance. Apart from dermal irritation (erythema, edema, eschar, desquamation, blanching and/or hyperkeratosis) noted at the dose site of the first female treated between Days 1 and 14, and the additional four females between Days 1 through 4, 6, and/or 7, and between Days 1 through 7, 8 and/or 12 at the male dose sites, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. Although all animals lost body weight by Day 1, all animals gained body weight by the end of the 14-day observation period, except for one male and one female, which returned to their initial body weights. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

Based on the result of these study the dermal LD_{50} of 2-Methyl-1,2-benzisothiazolin-3-one Technical is greater than 5000 mg/kg of body weight in male and female rats.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

For the acute toxicity by the oral administration route one study was identified as key study. The study was conducted in compliance with OECD Guideline 425.

Due to the physical chemical properties of the technical material, an acute inhalation study could not be conducted on MBIT. However, when the technical is formulated into an end-use formulation a study could be conducted (an acute inhalation study was conducted, for the US EPA, on commercial product - a formulation of 24% MBIT in 96% propylene glycol 400. Male and female rats were exposed to one dose (2.22 mg/L) of these commercial product - aerosol - for 4 hours, nose-only).

For the acute toxicity by the dermal administration route two studies were performed. Different results were obtained in these studies. For the precautionary reasons the first study (Cerven D.R. (2009b)) was identified as key study. This study was conducted in compliance with OECD Guideline 402. MBIT was applied to the shaved intact skin of male and female rats for 24 hours, occluded. Rats were observed for 14 days post treatment. All ten rats died within one day of the 5000 mg/kg dermal application. Three rats died by day 1 of the 2000 mg/kg dermal application. Since two of the five female rats survived the 2000 mg/kg dermal application, this indicated that the dermal LD_{50} was slightly below this dosage. The dermal LD_{50} of 2-methyl-1,2-benzisothiazol-3(2H)-one technical is less than 2000 mg/kg of body weight in rats (but judged greater than 200 mg/kg).

4.2.4 Comparison with criteria

Table 16: Presents the toxicological results in comparison with CLP criteria.

Toxicological result	**CLP criteria**
Oral LD_{50}, rat: 175 mg/kg	Cat. 3: $50 < LD_{50} \leq 300$ mg/kg (oral)
Inhalation LC_{50}: > 0.53 mg/L	Cat. 3: $0.5 < LC_{50} \leq 1.0$ mg/L (mist)
Dermal LD_{50}:> 200 mg/kg	Cat. 3: $200 < LD_{50} \leq 1000$ mg/kg (dermal)

4.2.5 Conclusions on classification and labelling

The acute oral toxicity of MBIT meets the CLP criteria. Based on the results of the acute oral toxicity study MBIT has to be classified for acute toxicity – oral route – in category 3 (Acute Tox. 3, H301) according to CLP criteria. The proposed Acute Toxicity Estimates (ATE) value, for oral route, is 175 mg/kg body weight.

The acute inhalation toxicity of MBIT meets the CLP criteria. Based on the results of the acute inhalation toxicity study of formulation of 24% MBIT, the MBIT has to be classified for acute toxicity – inhalation route – in category 3 (Acute Tox. 3, H331) according to CLP criteria. The proposed Acute Toxicity Estimates (ATE) value, for dermal route, based on the Table 3.1.2 of CLP Regulation, is 300 mg/kg body weight.

The acute dermal toxicity of MBIT (Cerven D.R. (2009b)) meets the CLP criteria. Based on the results of the acute dermal toxicity study MBIT has to be classified for acute toxicity – dermal route – in category 3 (Acute Tox. 3, H311) according to CLP criteria. The proposed Acute Toxicity Estimates (ATE) value, for inhalation route (mist), based on the Table 3.1.2 of CLP Regulation, is 0.5 mg/l.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

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

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

For determination of acute inhalation toxicity, pure MBIT could not be tested due to unspecified physical chemical properties of the technical material. Therefore, testing was performed using a commercial product with a formulation of 24 % MBIT in 96 % propylene glycol 400. Acute inhalation toxicity was tested in Sprague-Dawley rats (5 males and 5 females) according to the OECD TG 403 and US EPA 870.1300. The animals were exposed to the test substance in the form of aerosol during 4 h, nose only. The study was performed in accordance with GLP, and there were no deviations from the guideline. Only one dose level was tested, 2.22 mg/L of the product, thus 0.53 mg MBIT/L.

There was no mortality during the study. Clinical signs included activity decrease,

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

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

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

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

Comments received during public consultation

One Member State Competent Authority (MSCA) commented that the LC_{50} of > 0.53 mg/L is very close to the concentration limit for Cat. 2 of acute inhalation toxicity, and that as the study was performed with a formulation instead of the active substance, there are some uncertainties, which is why classification in Cat. 2 instead of Cat. 3 should be discussed. In addition, they recommended proposing an ATE value to facilitate uniform and reproducible classification of mixtures.

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

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

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

Assessment and comparison with the classification criteria

Oral route

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

Inhalation route

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

However, RAC is of the opinion that MBIT should be labelled with EUH071 ("Corrosive to the respiratory tract"). In the criteria, it is stated that EUH071 applies "For substances and mixtures in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I. For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled."

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

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

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

Dermal route

There were two studies on acute toxicity via the dermal route in the dossier. Both were performed according to the OECD TG 402 and considered reliable without restrictions by the DS. Mortality was seen in the study: by day 1, 10/10 of the rats died at 5 000 mg/kg bw. Furthermore, at 2 000 mg/kg bw, 3/5 of the

females died, also by day 1. In the interest of conserving animals, particularly since the test material is a strong irritant, no further dosing was conducted in this study. Therefore, the LD_{50} was considered to be slightly below 2 000 mg/kg bw based on the result in females. In the second study, no mortalities or signs of toxicity were seen either at 2 000 mg/kg bw or 5 000 mg/kg bw, apart from skin irritation at the higher dose. The LD_{50} was therefore determined to be > 5 000 mg/kg bw. There is not enough information in the dossier to explain the different outcomes of these two studies. Both were performed in the rat (first in Wistar, second in Fisher 344), and both sexes were included in both studies, except for the 2 000 mg/kg bw dose in Wistar, where only females were included (dosing was discontinued following mortality in females).

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

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

The hazard class STOT-SE has 3 categories, with Categories 1 and 2 being distinct from Category 3 in terms of the toxicity they cover and the criteria. Categories 1 and 2 for non lethal “significant and/or severe toxic effects” are the basis for classification with the category reflecting the dose level required to cause the effect. Category 3 covers “transient effects” occurring after single exposure, specially respiratory tract irritation (RTI) and narcotic effects (NE).

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

STOT SE 1 or 2

No toxicity to a specific organ in the absence of lethality was observed in acute oral, inhalation or dermal toxicity studies. Classification as STOT SE 1 or 2 is therefore not appropriate.

STOT SE 3

In acute inhalation toxicity study in rats no clinical signs indicating respiratory irritation were observed. The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities.

Additional information: two acute respiratory depression (RD_{50}) studies. They have not been conducted on MBIT, but on two structurally related compounds: octyl

isothiazolone (OIT, CAS Nr 26530-20-1) and methyl isothiazolone (MIT, CAS Nr 2682-20-4). The results of these tests are presented below.

Table 17: Summary of respiratory irritation data.

Parameters	Species	Results	Bibliographic references
Upper airway irritation RD_{50} TS: OIT Technical (99 % a.i.) GLP: Yes Deviation: No	Mouse Sex: Male	RD_{50} = 19.9 µg/L	Ulrich, 1991
Upper airway irritation RD_{50} TS: MIT Technical (98.6 % a.i.)	Mouse Sex: Male	Greater than 157 µg TS/L, the highest concentration tested	Hilaski, 1994

4.3.2 Comparison with criteria

STOT SE 1 or 2

There are no relevant data to compare with criteria.

STOT SE 3

OIT: the study - upper airway irritation test in mice - was conducted in accordance with US EPA Guideline 81-3. There were no guideline deviations. Seven groups of 4 male Swiss Webster mice were exposed once for 10 minutes using head-only exposure methods to aerosol atmospheres of Skane™ M-8 Technical in propylene glycol. The exposure concentrations ranged from 3.2 to 9.4 µg/L. The aerosol was characterized by a mass median aerodynamic diameter of approximately 1.8 microns (geometric standard deviation of 2.7). The group average respiratory rate was monitored before, during and after each exposure and the percent change in respiratory rate was calculated. The RD_{50} was calculated to be 19.9 µg/L.

MIT: the study - upper airway irritation test in mice - was conducted in accordance with ASTM Method E981-84 (Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals, American Society for Testing and Materials, Designation E981-84). There were no guideline deviations. No greater than 47 percent decrease in respiratory rate was achieved with the test material at the concentrations tested. The RD_{50} was greater than 157 µg/L, the highest concentration tested. Using the ASTM method E981-84 classifications for decreases in respiratory rate, the results of this study would be rated as a moderate response (20-50% decrease) for sensory irritation.

4.3.3 Conclusions on classification and labelling

Classification and labelling is not required.

Justification:

STOT SE 1 or 2:
No toxicity to a specific organ in the absence of lethality was observed in acute oral, inhalation or dermal toxicity studies. Classification as STOT SE 1 or 2 is therefore not appropriate.

STOT SE 3:
- in acute inhalation toxicity study in rats no clinical signs indicating respiratory irritation were observed. The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities,
- Guidance on the Application of the CLP Criteria: it is a reasonable assumption that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing frank respiratory tract corrosion. If there is evidence from animal studies or from human experience to support this then Category 3 may be appropriate. In general, a classification for corrosivity is considered to implicitly cover the potential to cause RTI and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier,
- Committee for Risk Assessment, Annex 1 Background document to the Opinion proposing harmonised classification and labelling at EU level of 2-methylisothiazol-3(2H)-one (ISO) - RAC note: the upper airway irritation test is a measure of sensory irritation and whilst it can be used for setting up workplace exposure limits, it is not used for classification purposes.

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

Summary of the Dossier Submitter's proposal

The DS did not propose classification, as there was no toxicity to a specific organ in the absence of lethality observed in the acute oral, inhalation and dermal toxicity studies, or there were no transient effects occurring after single exposure, especially for respiratory tract irritation and narcotic effects (respectively). The DS noted that the guidance on CLP Criteria (Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017) states that it is reasonable to assume that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing respiratory tract corrosion. However, in the acute inhalation toxicity study in rats, there were no clinical signs indicating respiratory irritation. The gross necropsy did not reveal any observable abnormalities related to respiratory irritation either.

As additional information in relation to STOT SE 3 (respiratory tract irritation), the DS presented data from two respiratory depression (RD) studies conducted with two structurally related compounds: octyl isothiazolinone (OIT, CAS 26530-20-1) and methyl isothiazolinone (MIT, CAS 2682-20-4). The upper airway irritation RD_{50} values (exposure concentration producing a 50 % respiratory rate decrease)

were calculated to be 19.9 µg/L for OIT and 157 µg/L for MIT. RD_{50} results are not available from MBIT. The DS did not propose to classify MBIT for STOT SE 3 noting that the upper airway irritation test is a measure of sensory irritation and whilst it can be used for setting up workplace exposure limits, it is not used for classification purposes.

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

Regarding STOT SE 3 (respiratory tract irritation), as already mentioned above, it is reasonable to assume that skin corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing respiratory tract corrosion. However, RAC is of the opinion that labelling MBIT with EUH071 ("Corrosive to the respiratory tract"), as proposed under acute toxicity, is more appropriate and adequately covers this hazard. Therefore, classifying for STOT SE 3; H335 ("May cause respiratory irritation") is not needed.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

To determine if MBIT causes skin corrosion/irritation the experimental study was performed. The study was conducted in compliance with OECD Guideline 404. There were no guideline deviations. Initially, one healthy New Zealand White rabbit was dosed with MBIT. The test article (0.5 g) was placed on the intact skin of the back (Site 1) and kept in contact with the skin for three minutes. Erythema and edema were

scored one hour following patch removal. Since the 3-minute exposure did not indicate a corrosive effect, two additional rabbits were added to the study. Al three animals were dosed at site #2 for an exposure period of 1 hour and at side #3 for an exposure period of four hours. Each site was scored for erythema and edema at 1, 24, 48 and 72 hours following patch removal and again on days 7 and 14. Animals were observed for systemic signs at each dermal scoring interval. A modified primary irritation index was calculated. Body weights were recorded present and at termination.

Table 18: Overview of experimental data on skin irritation.

Method	**Results**	**Remarks**	**Reference**
Test animals: Species: rabbit Strain: New Zealand White Sex: females OECD Guideline for Testing of Chemicals No. 404	Skin corrosive	1 (reliable) Key study Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Di Donato L.J. (2009)

On Day 14 one rabbit's skin had eschar, one rabbit's skin had necrosis and three rabbits had flaking skin. Severe erythema (4.0) was noted on Days 7 and 14. Edema was absent to very slight on Day 14. Under the conditions of this study, MBIT was considered to be a skin corrosive.

Table 19: Table for skin irritation study, rabbits, 1 hour exposure.

score (average animals investigated)	time	Erythema	Edema
average score Draize scores (0 to maximum 4)	60 min	1.0	3.0
	24 h	1.0	1.33
	48 h	0.67	1.67
	72 h	0.67	1.33
other times	7 days	3.0	1.0
	14 days	3.0	0.0
average score	24h, 48h, 72h	0.78	1.44
reversibility: *		No	Yes

Table 20. Table for skin irritation study, rabbits, 4 hour exposure.

score (average animals investigated)	time	Erythema	Edema
average score Draize scores (0 to maximum 4)	60 min	2.0	3.0
	24 h	1.33	2.0
	48 h	1.33	2.0
	72 h	1.33	1.66
other times	7 days	4.0	2.0
	14 days	4.0	0.66
average score	24h, 48h, 72h	1.33	1.89
reversibility: *		No	No
average time for reversibility		Not applicable	14 days

* c : completely reversible
n c : not completely reversible
n : not reversible

4.4.1.2 Human information

No other relevant information is available.

4.4.1.3 Summary and discussion of skin irritation

According to the results of the rabbit skin corrosion/irritation study MBIT is considered to be a skin corrosive.

4.4.1.4 Comparison with criteria

According to CLP requirements the substance is classified, on the basis of the results of animal testing, as skin corrosion category 1 if:

- produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. Corrosive reactions are typified by ulcers, bleeding, bloody scabs and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia and scars. Histopathology shall be considered to discern questionable lesions. Three subcategories are provided within the corrosive category: subcategory 1A - where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B - where responses are described following exposure

between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C - where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days. During the test performed on MBIT the responses occur after one hour exposures and after exposure between 1 hour and 4 hours and observations up to 14 days.

After 1-hour exposure: on Day 14 severe erythema was observed at two animals. Edema was very slight on Day 7 and absent on Day 14.

After 4-hour exposure: on Day 14 one rabbit's skin had eschar, one rabbit's skin had necrosis and three rabbits had flaking skin. Severe erythema (4.0) was noted on Days 7 and 14. Edema was absent to very slight on Day 14.

Under the conditions of this study, MBIT was considered to be a skin corrosive and should be classified, according to CLP Regulation, in subcategory 1B – responses occur after 1 hour exposure and observations up to 14 days.

4.4.1.5 Conclusions on classification and labelling

According to CLP regulation requirements 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) should be classified as Skin corrosive category 1B (Skin Corr. 1B) with hazard statement H314 (Causes severe skin burns and eye damage).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify MBIT as Skin Corr. 1B; H314. The dossier includes one study on skin corrosion/irritation, performed in the rabbit (New Zealand White, females) in compliance with OECD TG 404. There were no deviations from the guideline, and the study was considered reliable without restrictions. Initially, one rabbit was dosed 0.5 g of MBIT on the intact skin on the back at site 1, and MBIT was kept in contact with the skin for 3 minutes. Erythema and oedema were scored after one hour, and since the exposure did not indicate a corrosive effect, two additional rabbits were included. All three rabbits were dosed at site 2 for an exposure period of 1 hour, and at site 3 for an exposure period of four hours. All sites were scored for erythema and oedema at 1, 24 and 72 hours following patch removal, and furthermore on day 7 and 14. The results are summarised in the Table below. Human data for skin irritation/corrosion is not available.

Table. Summary of the results of the skin irritation study

		1 hour exposure		4 hour exposure	
	Time	Erythema	Oedema	Erythema	Oedema
Draize score (average, scale 0 – 4)	60 min	1.0	3.0	2.0	3.0
	24 h	1.0	1.33	1.33	2.0
	48 h	0.67	1.67	1.33	2.0
	72 h	0.67	1.33	1.33	1.66
	7 days	3.0	1.0	4.0	2.0

	14 days	3.0	0.0	4.0	0.66
	Further details from day 14	–		Eschar in 1/3, necrosis in 1/3, flaking skin in 3/3	
Reversibility		No	Yes	No	No

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

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

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

4.4.2 Eye irritation

4.4.2.1 Non-human information

2-methyl-1,2-benzisothiazol-3(2H)-one technical produced severe irritation/corrosive effects to the skin of rabbits (see section 4.4.1). Taking into account the corrosive

properties of MBIT observed in the Acute Dermal Irritation Study, the Acute Eye Irritation Study was not performed.

According to CLP Regulation skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). Also according to section 3.3.2.1.2.5 (Testing methods: *In vivo* methods) of Guidance on the application of CLP Criteria "Testing for eye irritation would not be carried out on substances known or predicted to be corrosive to skin. Such substances are automatically considered to be severely damaging to the eye and are classified but not labelled for serious eye damage in addition to skin corrosion".

Taking into account the above mentioned information Dossier Submitter recommends classification, according to CLP, of 2-methyl-1,2-benzisothiazol-3(2H)-one technical as corrosive to the eyes – Eye Dam. 1, H318.

4.4.2.2 Human information

No other relevant information is available.

4.4.2.3 Summary and discussion of eye irritation

2-methyl-1,2-benzisothiazol-3(2H)-one technical produced severe irritation/corrosive effects to the skin of rabbits (see section 4.4.1).

Dossier Submitter recommends also classification (see explanation in Section 4.4.2.1), according to CLP, of 2-methyl-1,2-benzisothiazol-3(2H)-one technical as corrosive to the eyes – Eye Dam. 1, H318.

4.4.2.4 Comparison with criteria

There are no relevant data to compare with criteria (No experimental studies were performed to assess the corrosive potential of substance to the eyes).

2-methyl-1,2-benzisothiazol-3(2H)-one technical produced severe irritation/corrosive effects to the skin of rabbits (see section 4.4.1).

Dossier Submitter recommends also classification (see explanation in Section 4.4.2.1), according to CLP, of 2-methyl-1,2-benzisothiazol-3(2H)-one technical as corrosive to the eyes – Eye Dam. 1, H318.

4.4.2.5 Conclusions on classification and labelling

According to CLP regulation requirements 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) should be classified for serious eye damage category 1 with hazard statement H318 (Causes serious eye damage).

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal The DS proposed to classify MBIT as Eye Dam. 1; H318, based on the Skin Corr. 1 classification. An acute eye irritation study has not been performed with MBIT due to the corrosive properties observed in the acute dermal irritation study. Comments received during public consultation The manufacturer was in agreement with the proposed classification. Also one MSCA supported the classification. Assessment and comparison with the classification criteria According to the CLP, skin corrosive substances shall be considered as leading to serious damage to the eyes. RAC agrees that based on the skin corrosion study, classification as Eye Dam. 1; H318; Causes serious eye damage, is justified. The hazard statement H318 will not be added in the labelling column. Indeed, where a chemical is classified as skin corrosion, labelling for serious eye damage/eye irritation can be omitted as this information is already included in the hazard statement for skin corrosion (see section above) (European Commission, 2016).

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available.

4.4.3.2 Human information

No relevant data.

4.4.3.3 Summary and discussion of respiratory tract irritation

No data available.

4.4.3.4 Comparison with criteria

There are no relevant data to compare with criteria.

4.4.3.5 Conclusions on classification and labelling

Classification and labelling is not required.

4.5 Corrosivity

See section 4.4.

4.5.1 Non-human information

See section 4.4.

4.5.2 Human information

See section 4.4.

4.5.3 Summary and discussion of corrosivity

See section 4.4.

4.5.4 Comparison with criteria

See section 4.4.

4.5.5 Conclusions on classification and labelling

See section 4.4.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

To determine if MBIT causes skin sensitisation three tests were performed.

Table 21: Overview of experimental data on skin sensitisation.

Method	Results	Remarks	Reference
Local lymph node assay (LLNA) Test animal: Species: mouse Test guideline: OECD 429	Sensitizer at EC_3 ≥ 10455 ppm a.i. (1.04%) [261 µg a.i./cm^2].	1 (reliable without restrictions) Key study Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.9% a.i.	McMillan, S. and Donald, E. (2008)
Local lymph node assay (LLNA) Test animal: Species: mouse Test guideline: OECD 429	Sensitizer at EC_3 = 6900 ppm (0.69%), [173 µg a.i./cm^2].	1 (reliable without restrictions) Key study Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.9% a.i.	Kirk M. (2009)
Buehler test Test animal: Species: guinea pigs	Sensitizer at 1800 ppm a.i. [229 µg a.i./cm^2].	1 (reliable without restrictions) Key study Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.9% a.i.	Hall D.A. (2009)

4.6.1.2 Human information

One human repeated insult patch test (HRIPT) study was conducted with MBIT in the 1970s. However, this study is considered as a non key study (Davies R.E., Coope K.B., Kynoch S.R. and Collins M.E. (1975)).

Healthy adult volunteers were selected for this study after consideration of any previous history of allergies and dermatitis (45 healthy adult human volunteers). Patches were applied every 2 days (Monday, Wednesday and Friday) for five weeks for a total of 15 applications. A fortnight after the fifteen induction applications, a challenge application was applied to both upper arms of each volunteer. A second challenge application was made 8 or 12 weeks later to volunteers showing evidence of possible sensitivity or atypical reactions in response to the first challenge. During the induction period the sites were observed 24 or 48 hours after patch removal. After the first and second challenge applications the sites were examined 24 and 72 hours after patch removal. The challenge application gave reactions in 16 volunteers which persisted, were atypical or were greater than during the induction period in one or more of the test materials and/or propylene glycol. A second challenge was carried out and in 14 of the 16 volunteers the propylene glycol was substituted by liquid paraffin. Second challenge: MBIT, 500 ppm: marked dermal reactions in 7 volunteers and mild skin responses in 2 individuals. It can be concluded that MBIT 500 ppm produced evidence of dermal sensitisation in 9 volunteers and are therefore considered to be sensitisers.

4.6.1.3 Summary and discussion of skin sensitisation

The first study study (McMillan, S. and Donald, E. (2008)) was conducted in compliance with OECD Guideline 429. There were no guideline deviations. MBIT was applied onto the dorsum of each mouse ear for 3 consecutive days. Three days later (day 6) each mouse received an intravenous injection of methyl-^{3}H-thymidine and 5 hours later the draining lymph nodes were collected and the incorporation of methyl-^{3}H-thymidine was assessed by scintillation counting.

Table 22: Detailed information including induction/scoring schedule for skin sensitisation test *(*McMillan, S. and Donald, E. (2008)).

Inductions	**LLNA**		**Observations/Remarks**
	Day of treatment	Application	
Induction	1, 2, 3	Topical	None
^{3}H-thymidine	6	Injection	None

Table 23: Result of LLNA sensitisation test *(*McMillan, S. and Donald, E. (2008)).

Treatment	**Measured dose**	**DPM (mean)**	**SI (Test/control Ratio)**	**Results**
Acetone/olive oil (4:1 v/v)	0 ppm	1206	1.0	Negative
MBIT	3000 ppm	2391	2.0	Negative
MBIT	10000 ppm	3466	2.9	Negative
MBIT	30000 ppm	8770	7.3	Positive
MBIT	100000 ppm	12556	10.4	Positive
MBIT	300000 ppm	18942	15.7	Positive
Acetone/olive oil (4:1 v/v)	0 ppm	1719	1	Negative
Hexylcinnamicaldehyde	10% (validation study 1)	4016	2.3	Negative
Hexylcinnamicaldehyde	25% (validation study 1), nominal	12283	7.1	Positive
Hexylcinnamicaldehyde	50% (validation study 1), nominal	17608	10.2	Positive
Acetone/olive oil (4:1 v/v)	0 ppm	1020	1	Negative
Hexylcinnamicaldehyde	10% (validation study 2), nominal	3964	3.9	Positive
Hexylcinnamicaldehyde	23% (validation study 2), nominal	9780	9.6	Positive
Hexylcinnamicaldehyde	43% (validation study 2), nominal	15149	14.9	Positive
DPM = disintegrations per minute. SI = stimulation index.				

The second study (Kirk M (2009)) study was also conducted in compliance with OECD Guideline 429. There were no guideline deviations. MBIT was applied onto the dorsum of each mouse ear for 3 consecutive days. Three days later (day 6) each mouse received an intraperitoneal injection of the thymidine analog 5-bromo-2'-deoxy-uridine (BrdU) and 5 hours later the auricular lymph nodes were isolated, single-cell suspensions of lymph node cells (LNC) were generated, and the LNC suspension was analyzed by flow cytometry for BrdU incorporation and the total number of LNC.

Table 24: Detailed information including induction/scoring schedule for skin sensitisation test (Kirk M (2009)*).*

Inductions	**LLNA**		**Observations/Remarks**
	Day of treatment	Application	
Induction	1, 2, 3	Topical	None
Thymidine analog 5-bromo-2'-deoxy-uridine (BrdU)	5	intraperitoneal	None

Table 25: Result of LLNA sensitisation test (Kirk M (2009)).

Treatment	**Measured dose**	**DPM (mean)**	**SI (Test/control Ratio)**	**Results**
Acetone/olive oil (4:1 v/v)	0 ppm	26834	1.0	Negative
HCA positive control	25 %	237378	8.8	Positive
MBIT	100 ppm	28474	1.1	Negative
MBIT	3000 ppm	70073	2.6	Negative
MBIT	10000 ppm	88831	3.3	Positive
MBIT	30000 ppm	200971	7.5	Positive
DPM = disintegrations per minute. SI = stimulation index.				

The third study was conducted in compliance with OECD 406 Method (Buehler test; Hall D.A. (2009)). There were no guideline deviations. 0.4 ml of MBIT or HCA positive control was applied to the shaved intact skin of the male and female guinea pigs using a 25 mm Hilltop chamber with a 20 mm cotton pad. The test sites were covered with a strip of rubber dental dam sufficient to cover the treated areas. The torso was wrapped with non-irritating tape to provide occlusion. After 6 hours, the dams and test articles were removed and the treated sites were cleansed with distilled water and dried with a surgical sponge or soft towelling.

Under the conditions of this study, 2-methyl-1,2-benzisothiazol-3(2H)-one, when tested at 600 ppm (76 µg/cm^2) and 1200 ppm (153 µg/cm^2) did not produce delayed contact hypersensitivity in guinea pigs.

When MBIT was tested at 1800 ppm (229 µg/cm^2), a minimal incidence (20%) of delayed contact hypersensitivity in guinea pigs was observed.

The positive control, α-Hexylcinnamaldehyde (HCA), technical, 85%, did produce delayed contact hypersensitivity in guinea pigs, which confirmed the validity of this test.

Table 26: Detailed information including induction/challenge/scoring schedule for skin sensitisation test (Buehler method) (Hall D.A. (2009)).

Inductions	**Day of Treatment**	**Observations/Remarks**
pretreatment	day 0	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 1	day 1	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 2	day 3	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 3	day 5	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 4	day 8	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 5	day 10	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 6	day 12	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 7	day 15	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 8	day 17	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Induction 9	day 19	MBIT: 600 ppm, 1200 ppm and 1800 ppm, No erythema noted
Challenge	day 33	Group 3, 1800 ppm MBIT: 24h: 2 guinea pigs = score of 1 and 1 guinea pig with score of 0.5; 48h: 1 guinea pig with score of 0.5
Re-challenge	day 40	Group 3, 1800 ppm MBIT: 24h: 2 guinea pigs = score of 1; 48h: no erythema noted
Induction 1	week 1 day 1	Positive control (HCA), undiluted 100 %, erythema was faint to absent
Induction 2	week 2 day 8	Positive control (HCA), undiluted 100 %, erythema was faint to moderate
Induction 3	week 3 day 15	Positive control (HCA), undiluted 100 %, erythema was faint to moderate

Test group	**Positive control (HCA)**
5	Induction: day 1, day 8, and day 15 and Challenge: day 29
6	Challenge: day 1

Table 27: Result of skin sensitisation test (Buehler) (Hall D.A. (2009)).

	Number of animals with signs of allergic reactions / number of animals in group		
Challenge	**Naive control (600 ppm, 1200 ppm and 1800 ppm MBIT)**	**Test group 3 (1800 ppm MBIT)**	**Positive control (HCA)**
scored after 24h	0/10	2/10	3/10
scored after 48h	0/10	0/10	3/10
Re-challenge			--
scored after 24h	0/3	2/2	--
scored after 48h	0/3	0/2	--

4.6.1.4 Comparison with criteria

A Stimulation Index (SI) equal to or greater than 3, which is necessary for a substance to be classified as skin sensitizer was achieved in two LLNA tests performed on MBIT.

In LLNA sensitisation test performed by McMillan (McMillan, S. and Donald, E. (2008)) the Stimulation Index was above 3 at MBIT concentration 1.04%, what fulfils the criteria for Skin Sens. 1A, EC3 value ≤ 2%.

In LLNA sensitisation test performed by Kirk (Kirk M (2009)) the Stimulation Index was above 3 at MBIT concentration 0.69%, what fulfils the criteria for Skin Sens. 1A, EC3 value ≤ 2%.

When 2-methyl-1,2-benzisothiazol-3(2H)-one was tested by using Buehler method at 0.18% (1800 ppm (229 µg/cm^2)), a minimal incidence (20%) of delayed contact hypersensitivity in guinea pigs was observed, what fulfils the criteria for Skin Sens. 1A (the substance is classified as skin sensitizer Category 1A if in Buehler test ≥ 15% of the animals should respond positively at ≤ 0.2% topical induction dose).

Setting of specific concentration limits (SCL):

According to the Guidance on the Application of the CLP criteria (Version 4.1, June 2015) SCLs for skin sensitisation can be set based on the results from animal testing as reported below. SCL are set on the basis of testing of the substance and never on the basis of testing of a mixture containing the sensitising substance. Setting of SCL is based on potency; potency is already considered for subcategorization defining generic concentration limits. SCL generally applies for the most potent skin sensitisers classified in 1A. The following schemes can be used for determination of potency categories for sensitisers. The potency categories given in the tables below (for skin sensitisation potency in the Mosue Local Lymph Node Assay and potency on basis of the Buehler assay) are described in Basketter et al. (2005)

Table 28: Skin Sensitisation Potency in the Mouse Local Lymph Node Assay.

EC3-Value (% w/v)	Potency	Predicted sub-category (*)	Concentration Limit (% w/v)
≤ 0.2	Extreme	1A	0.001 (SCL)
> 0.2 - ≤ 2	Strong	1A	0.1 (GCL)
> 2.0	Moderate	1B	1.0 (GCL)

Table 29: Potency on basis of the Buehler assay.

Concentration for intradermal induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Predicted sub-category	Concentration Limit (% w/v)
≤ 0.2	≥ 60	Extreme	1A	0.001 (SCL)
≤ 0.2	≥ 15 - < 60	Strong	1A	0.1 (GCL)
> 0.2 - ≤ 20.0	≥ 60	Strong	1A	0.1 (GCL)
> 0.2 - ≤ 20.0	≥ 15 - < 60	Moderate	1B	1.0 (GCL)
> 20.0	≥ 15	Moderate	1B	1.0 (GCL)

In order to determine into which category MBIT should be placed – extreme, strong or moderate sensitiser – the animal data is compared below with the criteria taken from the guidance:

In LLNA sensitisation test performed by McMillan (2008) the Stimulation Index was above 3 at MBIT concentration 1.04%, what fulfils the criteria for strong sensitiser.

In LLNA sensitisation test performed by Kirk (2009) the Stimulation Index was above 3 at MBIT concentration 0.69%, what fulfils the criteria for strong sensitiser.

When 2-methyl-1,2-benzisothiazol-3(2H)-one was tested by using Buehler method at 0.18% (1800 ppm (229 µg/cm^2)), a minimal incidence (20%) of delayed contact hypersensitivity in guinea pigs was observed, what fulfils the criteria for strong sensitiser.

By directly comparing the above criteria with the evidence presented for MBIT the appropriate potency classification for MBIT would be “strong” based on conduct of 2 LLNA studies and further supported by the Buehler assay.

Based on this potency classification the GCL of 0.1% would apply.

4.6.1.5 Conclusions on classification and labelling

Based on the following information:

- the results of two Local lymph node assay (LLNA) studies,

- the result of Buehler test,

- one human repeated insult patch test (HRIPT) study (additional supporting information)

it can be concluded that MBIT should be classified, according to CLP Regulation, as skin sensitizer (Skin Sens. 1A, H317 – May cause an allergic reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

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

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

Table. Stimulation indexes in the two LLNA studies.

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

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

In addition to the three key studies, as additional supporting information, the DS's proposal included one human repeated insult patch test (HRIPT) study

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

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

Comments received during public consultation

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

Three comments were received from MSCAs, all were in agreement with the proposed classification Skin Sens. 1A. On the matter of setting an SCL, one MSCA was in agreement with the proposed GCL of 0.1 %. One MSCA noted that, while the animal data does not support setting of a concentration limit of 0.001 % for extreme sensitiser, the high number of human volunteers (9/45) sensitised in the HRIPT study may justify the setting of an SCL. One MSCA was of the opinion that an SCL is necessary due to a steep increase in frequency of contact allergy by isothiazolinone compounds. Furthermore, they pointed out that MBIT is structurally closely related to 1,2-benzothiazil-3-one (BIT), and also methylisothiazolinone (MIT) and methylchloroisothiazolinone (CMIT). Therefore, it is reasonable to assume that if MBIT would show cross-reactivity to the other isothiazolinones, it would contribute significantly to the broad outcome of isothiazolinone allergy. Furthermore, they questioned why an SCL would be applied to BIT but not to MBIT, as LLNA data (included in the Annex 2, as well as in Table below) show MBIT to be a more potent sensitiser. Moreover, the sensitising capacity of MBIT is comparable to MIT, which was likewise considered based on LLNA data a "strong" sensitiser, but nevertheless an SCL was considered justified. Thus, as SCLs below the GCL were set for BIT, MIT and CMIT, they propose a lower SCL of 15 ppm also for MBIT.

Assessment and comparison with the classification criteria

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

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

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

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

	MBIT	BIT (CAS 2634-33-5)	MIT (CAS 2682-20-4)	CMIT/MIT (3:1) (CAS 55965-84-9)
LLNA result[a]	(1) EC_3 = 1.04 % (2) EC_3 = 0.69 % -> Skin Sens. 1A, strong	(1) EC_3 = 2.3 % (2) EC_3 = 32.4 % (2) EC_3 = 4.8 % (3) EC_3 = 10.4 % -> Skin Sens. 1(B, moderate; classified under DSD)	(1) EC_3 = 0.86 % -> Skin Sens. 1A, strong	(1) EC_3 = 0.003 % (2) EC_3 = 0.007 % -> Skin Sens. 1A
HRIPT result[b]	(3) 9/45 (20 %) volunteers showed dermal sensitization at 500 ppm (details lacking).	(3) 5/58 (9 %) at 725 ppm aq., 0/54 (0 %) at 360 ppm aq (details lacking).	1/116 (0.9 %) volunteers at 400 ppm and 1/210 (0.5 %) at 500 ppm. (No dose-response.)	–
SCL	This proposal: 0.0015 %	0.05 %	0.0015 %	0.0015 %

Studies	(1) & (2): see the Tbale above and the Background Document (3) Davies *et al.*, 1975	(1) Gerberick *et al.*, 2005 in Contact Dermatitis 16(4):157-202. (2) Botham *et al.*, 1991 in Contact Dermatitis 25(3):172-177 (3) Basketter *et al.*, 1999 in Contact dermatitis 40(3):150-154	(1): see RAC opinion for 2-methylisothiazol-3(2*H*)-one	(1) & (2): see RAC opinion for Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC No 220-239-6] (3:1)

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

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

In summary, RAC is of the opinion that an SCL is necessary for MBIT. For deriving the limit, starting with the exposure level used in the HRIPT test (500 ppm) is considered justified. However, 20 % of the volunteers were reported to become sensitised at this dose level, and therefore RAC is of the opinion that the SCL should be considerably lower than that, clearly less than a tenth of 500 ppm. Furthermore, there are published reports suggesting that a closely related isothiazolinone, BIT, may induce skin sensitisation in humans at already levels of 20–30 ppm in gloves. Overall, RAC concludes to classify MBIT as Skin. Sens. 1A with an SCL of 15 ppm (0.0015 %).

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No relevant data are available.

4.6.2.2 Human information

No relevant data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

There are no relevant data to discuss respiratory sensitisation.

4.6.2.4 Comparison with criteria

There are no relevant data to compare with criteria.

4.6.2.5 Conclusions on classification and labelling

No conclusion can be drawn on respiratory sensitisation potential.

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal The DS did not evaluate respiratory sensitisation, as there was no relevant data available. Comments received during public consultation No comments were received. Assessment and comparison with the classification criteria RAC concludes that MBIT cannot be classified for respiratory sensitisation due to lack of data.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 30: Overview of experimental data on repeated dose toxicity: oral.

Method	Results	Remarks	Reference
Repeated dose toxicity – 14-day oral (drinking water) rat Species: rat Sex: males and females Strain: Crl:CD(SD) Number of animals/per group: 6 rats/sex/group Duration of treatment: 14 days Frequency of exposure: 5 days per week, daily or other Test guideline: no guidelines available	No-observed-effect level (NOEL) = 250 ppm. No-observed-adverse-effect level (NOAEL) = 500 ppm. Clinical signs: test substance-related clinical observations consisted of dermal atonia in the 500, 1000 and 2000 ppm group males and 2000 ppm group females, thinness in the 2000 ppm group males and females, piloerection in the 1000 ppm group males and 2000 ppm	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Roper J.M. (2009a)

GLP: yes Concentration: test substance drinking water concentrations were 250, 500, 1000 and 2000 ppm for Groups 2-5, respectively. Clinical signs: clinical examinations were performed once daily, and detailed physical examinations were performed weekly during the study and on the days of necropsy. Mortality: all animals were observed twice daily for mortality and moribundity. Body weight: individual body weights were recorded twice weekly and on the days of necropsy. Food consumption: individual food consumption was recorded at least twice weekly. Water consumption: individual water consumption was recorded at least twice weekly. Haematology: not conducted. Clinical Chemistry: not conducted. Urinalysis: not conducted. Gross and histopathology: Macroscopic: no significant changes observed – all examined tissues from male and female rats: scheduled necropsy, found dead, euthanized moribund or in extremis. Histopathology: not conducted	group males and females, decreased defecation in the 500, 1000 and 2000 ppm group males and 2000 ppm group females and small feces in the 1000 and 2000 ppm group males and females. Clinical observations for male no. 11122 (Group 4, 1000 ppm) on study days 7 and 8 included dermal atonia, decreased defecation, small feces, thinness, wet yellow material on the urogenital area and dried yellow material on the urogenital and anogenital area. These observations, which correlated with findings of low food and water consumption throughout the study for this animal, are suggestive of dehydration due to poor palatability of mBIT. Mortality: male no. 11122 (Group 4, 1000 ppm) was found dead on study day 9. Body weight gain: decreases in body weight = 10 – 41.6% were observed at concentrations of MBIT of 1000 ppm and higher. Test substance-related low mean body weights were noted throughout the study for the 500, 1000 and 2000 ppm group males and 1000 and 2000 ppm group females when compared to the control group. Attenuation of effects on body weight gain was observed for the 500 ppm group males and 1000 ppm group females beginning with the interval from study day 7 to 10 and continuing to the end of the study, as demonstrated by mean body weight gains that were comparable to or higher than the control group. Lower cumulative mean body weight gains were noted for the 500 and 1000 ppm group males and 1000 ppm group females at the end of the study, and mean body weight losses were observed for the 2000 ppm group males and females throughout the study. The low mean body weights and low body weight gains and/or losses were considered adverse		

	at test substance drinking water concentrations ≥ 1000 ppm. Food consumption and compound intake (in drinking water): test substance-related low mean food consumption was observed for the 1000 and 2000 ppm group males and 2000 ppm group females compared to the control group throughout the study. Decreases in food consumption = 11 – 76% and water consumption = 24 – 83% were observed at concentrations of MBIT of 1000 ppm and higher. Food consumption in the 500 ppm group males and females and 1000 ppm group females was lower from study day 0-7, but was comparable to the control group by the end of the study. Water consumption for the 500, 1000 and 2000 ppm group males and females was lower throughout the study when compared to the control group. Gross and histopathology: all macroscopic findings noted were considered to be spontaneous and/or incidental in nature and unrelated to test substance administration. There were no test substance related macroscopic findings at the scheduled necropsies or for the male (Group 4, 1000 ppm) that was found dead on study day 9. Other: a concentration of 800 ppm MBIT was chosen for the high dose in the 90-day oral (drinking water) toxicity study in rats.		
Repeated dose toxicity – 90-day oral (drinking water) rat Species: rat Sex: male and females Number of animals/per group: 10 rats/sex/group Strain: Crl:CD(SD) Test guideline: OECD 408 GLP: yes Duration of treatment: 91 days Frequency of exposure: daily	NOAEL = 200 ppm, approximately 13 and 15 mg/kg/day for males and females, respectively. Clinical signs: there were no test substance-related clinical or macroscopic observations. Functional observational battery, locomotor activity, coagulation and ophthalmology parameters were unaffected by test substance administration.	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Roper J.M. (2009b)

Concentration: test substance drinking water concentrations were 50, 200 and 800 ppm for Groups 2-4, respectively. Actual test substance exposure was 3, 13 and 50 mg/kg/day for the 50, 200 and 800 ppm group males and 4, 15 and 60 mg/kg/day for the 50, 200 and 800 ppm group females, respectively. Clinical signs: clinical examinations were performed daily, and detailed physical examinations were performed weekly. Mortality: all animals were observed twice daily for mortality and moribundity. Body weight: individual body weights were recorded weekly. Final body weights were recorded on the day of the scheduled necropsy. Food consumption: food consumption was recorded weekly. Water consumption: water and test substance consumption were recorded weekly. Ophthalmoscopic examination: ophthalmic examinations were performed during study weeks -1 and 12. Haematology: yes number of animals: all animals time points: end of study, study week 13 Parameters: total leukocyte count (white cells), erythrocyte count (red cells), haemoglobin, haematocrit, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial thromboplastin time, reticulocyte count, differential leukocyte count and red blood cell morphology Clinical Chemistry: yes number of animals: all animals time points: end of study, study week 13	Mortality: one male in the 200 ppm group was found dead on study day 57. Microscopic changes observed in this rat included diffuse acute congestion and pulmonary hemorrhage, marked diffuse necrosis of the tracheal mucosa and multifocal hemorrhage in the thymus. These changes were considered likely due to inadvertent aspiration of the test drinking water and this death was not considered due to direct systemic toxicity of the test substance. Body weight gain: test substance-related decreased mean body weights and body weight gains were observed for the 200 ppm group males and 800 ppm group males and females. Changes in body weight were considered secondary to decreases in water consumption. Food consumption and compound intake: test substance-related low mean water consumption was observed for all test substance treated male groups and for the 200 and 800 ppm group female groups. Test substance-related decreased mean food consumption was observed for the 200 and 800 ppm group males throughout most of the study. Ophtalmoscopic examination: no ophthalmic lesions indicative of toxicity were observed in any of the test substance treated groups. All findings observed were typical in prevalence and appearance for laboratory rats of this age and strain. Haematology: there were no test substance-related alterations in coagulation parameters. There were no test substance-related alterations in hematology noted for the 50 and 200 ppm group males and females or the 800 ppm group females.		

Parameters: albumin, total protein, globulin, albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, and triglycerides. Organ Weights: yes organs: adrenals, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus, and uterus Gross and histopathology: yes all dose groups, complete necropsy organs examined microscopically: adrenals, aorta, bone with marrow, bone marrow smear, brain, cervix, epididymides, exorbital lacrimal glands, eyes with optical nerve, gastrointestinal tract, heart, kidneys, larynx, liver, lungs, lymph nodes, nasal turbinates, ovaries, pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, salivary glands, seminal vesicles, skeletal muscles, skin, spinal cord (cervical, thoracic, lumbar), spleen, testes, thymus, thyroid, trachea, urinary bladder, uterus, vagina, gross lesions. Other examination: functional observational battery and locomotor activity data were recorded for all animals during study week 12.	Red cell counts were 6.2% lower than control group in the 800 ppm group males, associated with 31.6% higher absolute reticulocyte counts. Statistically significantly higher mean corpuscular volume (MCV) corresponded to higher reticulocyte counts in the 800 ppm group males. A slight, but statistically significantly, higher mean corpuscular hemoglobin (MCH) was also evident in the 800 ppm group males. Alterations in hematologic parameters were not considered adverse because of the low magnitude of change (<10%) in red cell counts. Clinical chemistry: lower total protein and globulin levels and higher A/G ratio in the 200 and 800 ppm group males and higher urea nitrogen and phosphorus levels in the 800 ppm group females. These findings were considered secondary to poor nutritional and/or hydration status. Organ weights: higher mean relative brain weight and lower mean absolute liver weight in the 800 ppm group males. These changes were considered indicative to be manifestation of the lower mean final body weight and not due to direct systemic toxicity. Organ weights and final body weights were unaffected by test substance administration in the 50 and 200 ppm group males and females and the 800 ppm group females. Gross and histopathology: there were no definitive test substance related macroscopic findings at the scheduled necropsy. Minimal or mild submucosal edema and inflammation (mixed inflammatory cell infiltrations) were noted in the forestomach. Also in the forestomach, the limiting ridge was enlarged and thickened due to hyperplasia and hyperkeratosis. The lesions in the glandular mucosa also		

	consisted of submucosal edema and inflammation, but there were also focal erosions in the superficial mucosa. Microscopic changes were considered to represent an adverse local irritation of the stomach resulting from administration of the test substance. Other: neuromuscular observations were unaffected by test substance administration. There were no statistically significant differences when the test substance treated males and females were compared to the control group at the study week 12 evaluation. Locomotor activity patterns (total and ambulatory activity counts) were unaffected by test article administration. Test substance consumption for 0, 50, 200 and 800 ppm MBIT 13 weeks test period: 0, 3, 13, and 50 mg/kg/day for males and 0, 4, 15, and 60 mg/kg/day for females, respectively.		
Repeated dose toxicity – 90-day oral (dietary) dog study Species: dog Strain: Beagle Sex: males and females Test guideline: OECD 409 GLP: yes Number of animals per group: 4/sex/group Duration of treatment: 90 days Frequency of exposure: 4 hours per day, 7 days per week for 91-92 consecutive days through the day prior to the scheduled necropsy. Post exposure period: following 91 or 92 consecutive days of test diet administration, all animals were euthanized. Concentration Test: diet concentrations were 250, 750, and 2000 ppm, (Groups 2, 3 and 4 respectively). Concentration in vehicle 250,	NOEL and NOAEL = 750 ppm (MBIT exposures of 26 and 27 mg/kg/day for males and females, respectively) Clinical signs: clinical observations of thinness and/or dermal atonia, lower body weight gains (or body weight losses), and lower food consumption were noted for 2000 ppm group males and females and were considered secondary effects of the test substance due to poor palatability when administered in the diet at this concentration. Mortality: there were no test substance-related effects on survival. All animals survived to the scheduled necropsy. Body weight gain: there were no body weight effects. Food consumption and compound intake: there were no food consumption effects.	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Roper J.M. (2009c)

750 and 2000 ppm MBIT in acetone Clinical signs: clinical examinations were performed daily, and detailed physical examinations were performed weekly. Mortality: the animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Body weight: individual body weights were recorded weekly. Food consumption: food consumption was recorded daily and reported weekly. Water consumption: reverse osmosis treated (on site) drinking water, delivered by an automatic watering system, was provided ad libitum throughout the study period. Ophthalmoscopic examination: ophthalmic examinations were performed during study weeks -1 and 12. Haematology: yes number of animals: all animals time points: prior to the initiation of dose administration (study week -1) and during study week 13 Parameters: total leukocyte count (white cells), erythrocyte count (red cells), haemoglobin, haematocrit, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial thromboplastin time, reticulocyte count, red cell distribution width, and differential leukocyte count. Clinical Chemistry: yes number of animals: all animals time points: prior to the initiation of dose administration (study week -1) and during study week 13 Parameters: albumin, total protein, globulin, albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase,	Haematology: test substance-related hematologic changes included lower absolute lymphocyte counts in the 2000 ppm group females, but were not considered adverse. Clinical chemistry: there were no alterations in serum chemistry. Urinalysis: there were no alterations in urinalysis parameters. Organ weights: at the scheduled necropsy (study week 13), higher relative to body weight liver weights were noted for the 2000 ppm group males and females, and higher absolute and relative to body weight adrenal gland weights were noted for the 2000 ppm group females. Effects on organ weights were considered secondary to test substance-related effects on final body weights. Gross and histopathology: there were no macroscopic or microscopic changes that were considered to represent systemic toxicity related to ingestion of the test substance. Thymic atrophy/involution was observed microscopically in the 2000 ppm group and was considered to be stress related and secondary to decreases in body weight and food consumption as a result of the poor palatability of the test diet. In the stomach, hypertrophy of the mucus-secreting cells of the surface epithelium occurred in the 750 ppm group females and the 2000 ppm group males and females and was likely related to a local irritant effect of the test substance on the gastric mucosa. Neither the thymic or gastric changes were considered to represent direct systemic toxicity of the test substance, but rather secondary or adaptive responses of these tissues.		

alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, triglycerides, and sorbitol dehydrogenase. Urinalysis: yes number of animals: all animals time points: prior to the initiation of dose administration (study week -1) and during study week 13 Parameters: specific gravity, pH, urobilinogen, total volume, color, clarity, protein, glucose, ketones, bilirubin, occult blood, leucocytes, nitrites and microscopy of sediment. Organ Weights: Yes, selected organs were weighed at the scheduled necropsy. organs: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyropid with parathyroid and uterus. Gross and histopathology: yes Necropsy: all dose groups. Selected tissues were examined microscopically from all animals. organs collected and placed in 10% neutral-buffered formalin: adrenals, aorta, bone with marrow, bone marrow smear, brain, cervix, epididymides, eyes with optical nerve, gall bladder, gastrointestinal tract, heart, kidneys, larynx, liver, lungs, lymph nodes, nose, ovaries, oviducts, pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, salivary glands, skeletal muscles, skin, spinal cord (cervical, thoracic, lumbar), spleen, testes, thymus, thyroid/parathyroid, tongue, , trachea, urinary bladder, uterus, ureters, vagina, gross lesions.			

Repeated dose toxicity - 4-week oral (dietary) dog Species: dog Strain: Beagle Sex: males and females Number of animals per group: 2 male and 2 female dogs per group Test guideline: no guidelines available GLP: Yes Duration of treatment: 4 weeks Frequency of exposure: 4 hours per day 7 days per week for 28 days Concentration in vehicle (Acetone): test diet concentrations were adjusted weekly as follows. During study week 0, Groups 1-4 received diets containing 0, 2000, 4000 and 8000 ppm of MBIT, respectively. During study week 1, Groups 1-4 received diets containing 0, 2000, 1000 and 3000 ppm of MBIT, respectively. During study week 2, Groups 1-4 received diets containing 0, 2000, 1000 and 500 ppm of MBIT, respectively, and during study week 3, Groups 1-4 received diets containing 0, 2000, 1000 and 2500 ppm of MBIT, respectively. Clinical signs: clinical examinations were performed daily, and detailed physical examinations were performed weekly. Clinical pathology evaluations (hematology, coagulation and serum chemistry) were performed prior to the initiation of dose administration (study week-3) and during study week 4. Complete necropsies were conducted for all animals. Mortality: the animals were observed twice daily for mortality and moribundity. Body weight: individual body weights were recorded weekly. Food consumption: food consumption was recorded daily and reported weekly. Water consumption: reverse osmosis-treated (on site) drinking	No overt toxicity was noted at the dietary concentrations used on this study (500, 1000, 2000, 2500, 3000, 4000 and 8000 ppm). Clinical signs: there were no test substance-related clinical observations. Mortality: all animals survived to the scheduled necropsy. Body weight gain: lower body weights, lower body weight gains and/or higher body weight losses, secondary to poor palatability of the test diets, were observed for all test diet-treated groups during study week 0 to 1. Food consumption and compound intake: lower initial palatability of the formulated diets for Group 2 (2000 ppm) and poor palatability of the 4000 and 8000 ppm diets formulated for Groups 3 and 4, respectively, resulted in lower food consumption and consequently, lower body weight gains and/or body weight losses. Haematology: there were no test substance-related effects on hematology parameters in the female test diet-treated groups. Potentially test substance-related, non-adverse, lower white blood cell and reticulocyte counts (percent and absolute) compared to the control group were noted in the Group 4 males at the study week 4 evaluation. Mean and individual white blood cell and reticulocyte counts in these animals were also noted to be lower compared to their respective pretest values. The significance of these findings is uncertain, as no other remarkable erythrocyte or leukocyte alterations were noted and there was no direct correlation to the concentration of the test substance in the diet. Clinical chemistry: there were no test substance-related effects	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Roper J.M. (2009d)

water, delivered by an automatic watering system, was provided ad libitum throughout the study period. Ophthalmoscopic examination: eyes were examined macroscopically at necropsy. Haematology: Yes number of animals: all animals time points: were performed prior to the initiation of dose administration (study week-3) and during study week 4. Parameters: total leukocyte count (white cells), erythrocyte count (red cells), haemoglobin, haematocrit, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial thromboplastin time, reticulocyte count, and differential leukocyte count. Clinical Chemistry: yes number of animals: all animals time points: were performed prior to the initiation of dose administration (study week-3) and during study week 4. Parameters: albumin, total protein, globulin, albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, and triglycerides. Gross and histopathology: Yes Complete necropsy was conducted for all animals during study week 4. Animals were euthanized by an intravenous injection of sodium pentobarbital and exsanguinated. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including contents. No tissues were	on serum chemistry parameters. Gross and histopathology There were no test substance-related macroscopic observations		

collected or saved following gross examination and the carcasses were discarded.			

Repeated dose toxicity – 14-day oral (drinking water) rat:

This study was conducted as a range-finding study for the 90-day rat oral (drinking water) toxicity study. Male and female rats were exposed to MBIT in drinking water daily for 14 days. No TS analyses were performed as part of this study. Beginning on study day 10, any water consumption value that was greater than 70 g/animal/day was considered erroneous and was removed from the data. Due to excessive toxicity, all Group 5 animals were euthanized at an interim necropsy on study day 11. The remaining animals in Groups 1-4 were euthanized following 14 days of test substance administration.

Complete necropsies were conducted for all animals found dead and at the scheduled necropsies. The gastric mucosa was carefully examined for ulcers or other signs of erosion. Following the macroscopic examination, the carcasses were discarded.

These concentrations (1000 ppm and 2000 ppm) were judged too high and would not be tolerated over a 90-day oral toxicity study. The rats were fasted overnight prior to blood collection.

Due to body weight losses, low food and water consumption and clinical observations of dermal atonia, decreased defecation, thinness and piloerection during the first 10 days of test substance administration, all 2000 ppm group males and females were submitted for an interim necropsy on study day 11. All animals in Groups 1-4 survived to the scheduled primary necropsy.

Systemic effects of MBIT administered in the drinking water to Crl:CD(SD) rats for up to 14 days were observed at drinking water concentrations of 1000 and 2000 ppm as evidenced by lower body weight gains and/or losses, lower food and water consumption and clinical observations consistent with dehydration and poor nutrition (dermal atonia, thinness, decreased defecation and small feces). Less severe effects on these same parameters were noted for the 500 ppm group males and were not considered to be adverse. The clinical observations are suggestive of dehydration, poor nutrition and stress, which is likely due to poor palatability of the test substance.

Therefore, based on the results of this range-finding oral (drinking water) study, the no observed effect level (NOEL) for oral (drinking water) administration of MBIT to Crl:CD(SD) rats for up to 14 consecutive days was 250 ppm. The no observed adverse effect level (NOAEL) was 500 ppm.

Based on the results of this range-finding study, decreases in body weight (10 – 41.6%), water consumption (24 – 83%) and food consumption (11 – 76%) were observed at concentrations of MBIT of 1000 ppm and higher. These concentrations

(1000 ppm and 2000 ppm) were judged too high and would not be tolerated over a 90-day oral toxicity study. A concentration of 800 ppm MBIT was chosen for the high dose in the 90-day oral (drinking water) toxicity study in rats

Repeated dose toxicity – 90-day oral (drinking water) rat:

This study was conducted in compliance with OECD 408 Guideline. There were no guideline deviations. Following 91 days of dose administration, all rats were euthanized. Blood samples for clinical pathology evaluations (hematology, coagulation and serum chemistry) were collected from all surviving animals just prior to the scheduled necropsy (study week 13). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals. Functional observational battery (FOB) assessments were recorded for all animals during study week 12. Locomotor activity was assessed for all animals during study week 12. The animals were fasted overnight prior to blood collection. The mean amounts of MBIT consumed (mg/kg/day) by each sex per dose group were calculated from the mean water consumed (g/kg/day) and the appropriate target concentration of test substance in the vehicle (ppm).

There were no test substance-related clinical or macroscopic observations. Functional observational battery, locomotor activity, coagulation and ophthalmology parameters were unaffected by test substance administration.

One male in the 200 ppm group was found dead on study day 57. Microscopic changes observed in this rat included diffuse acute congestion and pulmonary hemorrhage, marked diffuse necrosis of the tracheal mucosa and multifocal hemorrhage in the thymus. These changes were considered likely due to inadvertent aspiration of the test drinking water and this death was not considered due to direct systemic toxicity of the test substance.

Test substance-related low mean water consumption was observed for all test substance-treated male groups and for the 200 and 800 ppm group female groups. The test substance-related effect on water consumption was considered to be the result of poor palatability of the test substance in drinking water formulations and not due to direct systemic toxicity.

Test substance-related decreased mean body weights and body weight gains were observed for the 200 ppm group males and 800 ppm group males and females. At the end of the test substance administration period, the mean body weights were 7.2% lower than the concurrent control group mean for the 200 ppm group males, and 13.4% and 6.5% lower for the 800 ppm group males and females, respectively. Test substance-related decreased mean food consumption was observed for the 200 and 800 ppm group males throughout most of the study. Lower food consumption was consistent with the observed lower body weight gain in the 200 and 800 ppm group males.

Lower total protein and globulin levels and higher A/G ratio in the 200 and 800 ppm group males and higher urea nitrogen and phosphorus levels in the 800 ppm group females were attributed to poor nutritional and/or hydration status.

A lower mean final body weight was observed for the 800 ppm group males. Higher mean relative brain weight and lower mean absolute liver weight in the 800 ppm group males were considered to be manifestations of the lower mean final body weight and not indicative of

direct systemic toxicity period.

Treatment-related microscopic changes were observed in a few females from the 800 ppm group. The treatment-related changes were observed in the both the forestomach and glandular areas of the stomach. Minimal or mild submucosal edema and inflammation (mixed inflammatory cell infiltrations) were noted in the forestomach. Also in the forestomach, the limiting ridge was enlarged and thickened due to hyperplasia and hyperkeratosis. The lesions in the glandular mucosa also consisted of submucosal edema and inflammation, but there were also focal erosions in the superficial mucosa. Minimal hyperplasia of the surface epithelium adjacent to the erosions was also noted. Microscopic changes were considered to represent an adverse local irritation of the stomach resulting from administration of the test substance. There were no other treatment-related changes in the tissues/organs at any dose level.

Repeated dose toxicity – 90-day oral (dietary) dog study

The study was conducted in compliance with OECD Guideline Section 409 with analytical confirmation of TS concentrations in the diet. There were no guideline deviations. MBIT was administered as dietary admixtures offered for approximately 4 hours per day (not to exceed 4 hours), 7 days per week, for a minimum of 90 days to 3 groups (Groups 2-4) of Beagle dogs. A complete necropsy was conducted on all dogs.

There were no test substance-related effects on survival; however, one 2000 ppm group female was removed from the study and transferred to the WIL stock colony on study day 13 due to persistent inappetence and profound body weight loss requiring fluid supplementation. In the absence of significant exposure to the test substance, this animal's condition was attributed to poor palatability of the test diet at a concentration of 2000 ppm. All other animals survived to the scheduled necropsy.

There were no ophthalmic findings, clinical observations, body weight or food consumption effects, or alterations in serum chemistry or urinalysis parameters that were directly related to toxicity of the test substance; however, clinical observations of thinness and/or dermal atonia, lower body weight gains (or body weight losses), and lower food consumption were noted for 2000 ppm group males and females and were considered secondary effects of the test substance due to poor palatability when administered in the diet at this concentration.

Test substance-related hematologic changes included lower absolute lymphocyte counts in the 2000 ppm group females, but were not considered adverse.

At the scheduled necropsy (study week 13), higher relative to body weight liver weights were noted for the 2000 ppm group males and females, and higher absolute and relative to body weight adrenal gland weights were noted for the 2000 ppm group females. Effects on organ weights were considered secondary to test substance-related effects on final body weights.

There were no macroscopic or microscopic changes that were considered to represent systemic toxicity related to ingestion of the test substance. Thymic atrophy/involution was observed microscopically in the 2000 ppm group and was considered to be stress-related and secondary to decreases in body weight and food consumption as a result of the poor palatability of the test diet. In the stomach, hypertrophy of the mucus-secreting cells of the surface epithelium occurred in the 750 ppm group females and the 2000 ppm group males and females and was likely related to a local irritant effect of the test substance on the gastric mucosa. Neither the thymic or gastric changes were considered to represent direct systemic toxicity of the test

substance, but rather secondary or adaptive responses of these tissues.

Repeated dose toxicity - 4-week oral (dietary) dog

Lower initial palatability of the formulated diets for Group 2 (2000 ppm) and poor palatability of the 4000 and 8000 ppm diets formulated for Groups 3 and 4, respectively, resulted in lower food consumption and consequently, lower body weight gains and/or body weight losses.

Body weight gains in Groups 2 and 3 were comparable to the control group during study week 3 to 4 due to the development of tolerability to the diet and the reduction of the test substance concentration in Group 3 to a level below 2000 ppm. Body weight changes in Group 4 were comparable to the control group during study week 2 to 3 with the reduction of the test substance concentration to 500 ppm, but body weight losses were noted again during study week 3 to 4 due to poor palatability and low food consumption resulting from an increase in the test substance concentration to 2500 ppm.

Test substance dietary concentrations greater than 2000 ppm were not considered to be palatable. Groups 3 (4000 ppm during study week 0 to 1) and 4 (8000, 3000 and 2500 ppm in study weeks 0 to 1, 1 to 2 and 3 to 4, respectively) were noted with significantly decreased food consumption when the test substance concentrations in the diet were greater than 2000 ppm. Discontinuing administration of the test diet and returning to the basal diet during study days 3 through 6 for Groups 3 and 4 and study days 9 through 13 for Group 4 resulted in immediate increases in food consumption to a level comparable with the control group indicating changes in food consumption were due to poor palatability of the test diet rather than a direct toxic effect. Food consumption in Group 2 (2000 ppm) was slightly lower than the control group during study weeks 0 to 1, 1 to 2 and 2 to 3, but tolerability to the diet developed and food consumption values for these animals during study week 3 to 4 were comparable to the control group.

4.7.1.2 Repeated dose toxicity: inhalation

Table 31: Overview of experimental data on repeated dose toxicity: inhalation.

Method	Results	Remarks	Reference
Subchronic inhalation toxicity study in rats – 90 days Species: rat Strain: Crl:CD(SD) Sex: males and females Number of animals per group: 10 males and 10 females per group Frequency of exposure: 5 days per week, 6 hours per day Postexposure period: none, animals were necropsied the morning following the final exposure. Concentration: Target concentrations: 0, 0.02, 0.15, 0.7 or 7 mg MBIT/m^3 air Analytically determined concentrations: 0, 0.04, 0.19, 0.75, or 7.04 mg MBIT/m^3 air Particle size: the test aerosols were targeted to have average mass median aerodynamic diameters (MMAD) less than 3 microns (µm) Type of exposure: nose only Test guideline: OECD 413 GLP: yes Solid MBIT test material was dissolved in water at a concentration of 0.5% or 1.5% (5 or 15 mg/ml, respectively) and delivered as a liquid aerosol. The 0.5% test material solution was used for the 0.02 and 0.15 mg/m^3 exposure chambers and the 1.5% test material solution was used for the 0.7 and 7 mg/m^3 exposure chambers [analytically-determined aerosol concentrations of 0.0 ± 0.0, 0.04 ± 0.01, 0.19 ± 0.04, 0.75 ± 0.14, and 7.04 ± 0.95 mg MBIT/m^3 air (study mean ± standard deviation)]. The average mass median aerodynamic diameter (MMAD) of the liquid aerosol droplets in each of the exposure chambers were 1.87±1.50, 2.92±1.44, 1.96±2.12, and 2.61±1.48 microns (MMAD ±	The no-observed-effect-concentration (NOEC) of MBIT for Crl:CD(SD) rats of either sex, exposed via inhalation for six hours/day, five consecutive days/week for 13 weeks (65 exposures) was 0.19 mg/m^3. Detailed Clinical Observations: Examinations performed on all animals pre-exposure and weekly throughout the study revealed no treatment-related findings. Mortality: there were no treatment-related mortalities. Two rats however, did not survive the full duration of the study. One female rat in the 0.04 mg/m^3 exposure group was euthanized on day 86 for animal welfare reasons due to an accidental fracture of the nasal septum. One female rat in the 0.19 mg/m^3 exposure group was reported to be found dead with its head bent under in the nose-cone during exposure on day 33. The loss of this animal was attributed to an accidental death due to improper head position in the nose cone leading to possible asphyxiation. Body weight gain: mean body weight values for males exposed to 7.04 mg/m^3 were decreased throughout the study when compared to control animals. However, this decrease was statistically significant only on test days 47 and 54. Mean body weight values for females exposed to 7.04 mg/m^3 were also decreased relative to control animals throughout the study, with the decreases demonstrating statistical significance in all instances. Body weight gains for animals in these exposure groups were	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 98.34% a.i.	Krieger, S. M. and Thomas, J. (2012)

geometric standard deviation; GSD) for the 0.04, 0.19, 0.75, and 7.04 mg MBIT/m^3 exposure chambers, respectively. Clinical signs: Yes, one cage-side examination per day and one detailed clinical observation per day for the 13 weeks of exposure. Mortality: Yes, daily Body weight: Yes, pre-exposure, twice during the first week, and once per week thereafter. Food consumption: Yes, weekly Ophthalmoscopic examination: Yes, pre-exposure and prior to scheduled necropsy. Haematology: Yes, 10/sex/group at 13 week necropsy Parameters: Hematocrit, hemoglobin, red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, platelet (PLT) count, reticulocyte (RET) count, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC). Clinical Chemistry: Yes, 10/sex/group at 13 week necropsy Parameters: glucose, total cholesterol, blood urea nitrogen, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP), Gamma glutamyl transpeptidase (GGT), Electrolytes (NA, K, PHOS, CL and CA), trigylcerides, globulin, albumin/globulin ratio. Urinalysis: Yes, Color, appearance, specific gravity (refractometer) and urine volume, pH, Bilirubin, Glucose, Protein, Ketones, Blood, Urobilinogen.	also similarly decreased relative to control values. There were no statistically identified differences in the body weights of any other treated groups when compared to their respective controls. The treatment-related reduction in body weight gain, feed consumption and terminal body weights in male and female rats exposed to the highest MBIT concentration (7.04 mg/m^3) were likely due to the irritant effects of repeated inhalation exposure to this isothiazolone. Decreased body weight gain, feed consumption and terminal body weight were also reported in male and female rats similarly exposed to 20.5 mg/m^3 for two weeks (Krieger and Thomas (2012). Reduced body weight, in the absence of systemic toxicity, at inhaled concentrations that resulted in portal of entry lesions in the respiratory tract have been reported for other inhaled irritants such as acrylic acid (Miller et al.,1981) and H_2S (Dorman et al., 2004). Food consumption: mean food consumption values for females exposed to 7.04 mg/m^3 were statistically identified as decreased relative to controls throughout the study. Mean food consumption values for males exposed to 7.04 mg/m^3 were statistically identified as decreased relative to controls for the first three weeks of the study. There were no significant differences in the amount of feed consumed by any other treated groups when compared to their respective controls. Ophtalmoscopic examination: examinations performed on all animals pre-exposure and at termination revealed no treatment-related findings. Haematology: there were no statistically significant or treatment-related changes in any of the hematologic parameters		

Organ Weights: Yes organs: liver, kidneys, adrenals, gonads, heart, lung, spleen, brain, testes, epidiymides, ovaries, uterus, thymus, and thyroids at 13 week necropsy. Gross and histopathology: Yes. Gross and histopathology, all dose groups immediately after 13 week exposure. Histopathology: A complete necropsy was conducted on all animals. The necropsy included an examination of the external tissues and all orifices. Statistics: Body weights, urine volume, urine specific gravity, organ weights, clinical chemistry data, coagulation and appropriate hematologic data, was evaluated by Bartlett's test (alpha= 0.01; Winer, 1971) for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric (Steel and Torrie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA). If significant at alpha = 0.05, the ANOVA was followed respectively by Dunnett's test (alpha = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum test (alpha = 0.05; Hollander and Wolfe, 1973) with a Bonferroni correction (Miller, 1966) for multiple comparisons to the control. The experiment-wise alpha level was reported for these two tests. Descriptive statistics only (means and standard deviations) were reported for body weight gains, globulin, albumin/globulin ratio, RBC indices, differential WBC counts, chamber concentration, temperature, relative humidity, and airflow. Statistical outliers were identified by a sequential test (alpha = 0.02; Grubbs, 1969), but routinely excluded only from feed consumption. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons. Because numerous measurements were statistically compared in the	of males or females in any of the exposure groups as compared to their respective controls. Differential white blood cell counts were also unaffected by exposure to MBIT. Clinical chemistry: there were no statistically significant or treatment-related changes in any of the clinical chemistry parameters of males or females in any of the exposure groups as compared to their respective controls. Urinalysis: there were no statistically significant or treatment-related changes in mean urine volume or specific gravity in males or females in any of the exposure groups as compared to those of the controls. All other urinary parameters of males and females of all exposure groups were similar to those of the controls with no toxicologically relevant effects attributed to exposure to MBIT. Microscopic evaluation of the kidneys and the urinary bladder also did not reveal any treatment-related histopathological changes in males or females of the high-concentration groups. Organ weights: the terminal fasting body weight of males exposed to 7.04 mg/m^3 was 10.3% lower (not statistically identified) than that of the control males and was interpreted to be treatment related. Mean absolute lung weight of males exposed to 7.04 mg/m^3 was significantly lower (28.6%) than that of the controls and was interpreted to be secondary to the decrement in the body weight. While there was no change in the absolute testes weights of males exposed to 7.04 mg/m^3, their mean testes weight relative to body weight was significantly higher than that of the control. The higher relative testes weight was interpreted to be secondary to		

same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.	the lower body weights of males exposed to 7.04 mg/m^3. None of these organ weight changes were associated with any treatment-related histopathological alterations. Terminal fasting body weights of females exposed to 7.04 mg/m^3 was 14.8% lower than that of the controls, the difference was statistically significant and was interpreted to be treatment-related. Mean absolute liver weights and absolute ovary weights of females exposed 7.04 mg/m^3 were significantly lower (15% and 21.6%, respectively) than those of the controls. The lower absolute weights of the liver and ovaries were interpreted to be secondary to the decrement in body weight. Mean relative kidney weights relative and brain weights relative to body weights of females exposed to 7.04 mg/m^3 were significantly higher (12% and 15%, respectively) than those of the controls and were interpreted to be secondary to the lower mean body weight as compared to that of the controls. Relative adrenal weights of females exposed to 0.19 or 7.04 mg/m^3 were statistically identified as higher than that of the controls. However, they were interpreted to be spurious and unrelated to treatment due to lack of a dose-response relationship. None of these organ weight changes were associated with any treatment-related histopathological alterations. There were no treatment-related effects in the terminal body weights or in any of the organ weights of males and females exposed to ≤ 0.75 mg/m^3.		

To evaluate the potential for local and systemic toxicity of inhaled MBIT, groups of ten male and ten female Crl:CD(SD) rats were exposed via nose-only inhalation to liquid aerosols of MBIT six hours/day, five consecutive days/week for 13 weeks (a total of 65 exposures). Target exposure concentrations of 0, 0.02, 0.15, 0.7, and 7 mg/m^3 were selected based on the results of a 2-week range-finding study. Solid

MBIT test material was dissolved in water at a concentration of 0.5% or 1.5% (5 or 15 mg/ml, respectively) and delivered to the rats as a liquid aerosol. Analytically-determined exposure concentrations were based on the mass concentration of MBIT present in the test aerosol, not the mass concentration of the liquid aerosol *in toto.* The rats were exposed to analytically-determined aerosol concentrations of 0.0 ± 0.0, 0.04 ± 0.01, 0.19 ± 0.04, 0.75 ± 0.14, and 7.04 ± 0.95 mg MBIT/m^3 air (study mean ± standard deviation). The average mass median aerodynamic diameter (MMAD) of the liquid aerosol droplets in each of the exposure chambers were 1.87±1.50, 2.92±1.44, 1.96±2.12, and 2.61±1.48 microns (MMAD ± geometric standard deviation; GSD) for the 0.04, 0.19, 0.75, and 7.04 mg MBIT/m^3 exposure chambers, respectively. In-life observations, feed consumption, body weights, ophthalmology, urinalysis, coagulation, hematology, clinical chemistry, and organ weights were evaluated. In addition, a gross necropsy was conducted with extensive histopathologic examination of tissues. There were no treatment-related changes in in-life observations, ophthalmology, hematology, coagulation (prothrombin time), urinalysis, or gross pathological observations at the scheduled necropsy. Mean body weight values for males exposed to 7.04 mg/m3 were decreased throughout the study when compared to control animals (statistically significant only on test days 47and 54). Mean body weight values for females exposed to 7.04 mg/m^3 were statistically identified as decreased relative to control animals throughout the study. Body weight gains for animals in these exposure groups were also similarly decreased relative to control values. Mean feed consumption values for females exposed to 7.04 mg/m^3 were statistically identified as decreased relative to controls throughout the study. Mean feed consumption values for males exposed to 7.04 mg/m^3 were statistically identified as decreased relative to controls for the first three weeks of the study. Males and females exposed to 7.04 mg/m^3 had treatment-related lower terminal body weights (10.3% and 14.8%, respectively) as compared to those of the controls. Mean absolute lung weight of males exposed to 7.04 mg/m^3 was significantly lower (28.6%) and mean relative testes weights were significantly higher than those of the controls. Mean absolute liver weights and absolute ovary weights of females exposed 7.04 mg/m^3 were significantly lower (15% and 21.6%, respectively) and mean relative kidney weights and relative brain weights of females exposed to 7.04 mg/m^3 were significantly higher (12% and 15%, respectively) than those of the controls. All these organ weight changes were interpreted to be secondary to the lower terminal body weights of males and females exposed to 7.04 mg/m^3 and were not associated with any treatment-related histopathological changes.

The treatment-related reduction in body weight gain, feed consumption and terminal body weights in male and female rats exposed to the highest MBIT concentration (7.04 mg/m^3) were likely due to the irritant effects of repeated inhalation exposure to this isothiazolone. In males and females exposed to 0.75 or 7.04 mg/m^3 treatment-related histopathological changes were confined to the anterior nasal cavity and anterior larynx consistent with localized portal of entry irritant effects of the test material at the point of contact with the upper respiratory tract. There was no histopathological evidence of any primary systemic toxicity. In the anterior nasal cavity, males and females exposed to 7.04 mg/m^3 had bilateral, very slight or slight hyperplasia and hypertrophy of mucous cells mainly in the respiratory mucosa lining the middle to lower aspect of the anterior nasal septum, extending ventrally along the vomeronasal organ, the ventral meatus and variably involving the respiratory epithelium dorsal to the incisive ducts in some rats. In the anterior larynx of males and

females exposed to 0.75 or 7.04 mg/m^3, dose-dependent, very slight, slight or moderate squamous metaplasia of the surface epithelium was noted at the base of the epiglottis. A very slight or slight squamous metaplasia of the submucosal seromucinous glands was noted in males and females exposed to 7.04 mg/m^{3}and in one male exposed to 0.75 mg/m^3. Males and females exposed to 7.04 mg/m^3 and one male exposed to 0.75 mg/m^3 also had a very slight multifocal cystic atrophy of the seromucinous glands. Males and females exposed to 0.75 or 7.04 mg/m^3 had a very slight or slight, subacute to chronic inflammation of the mucosa at the base of the epiglottis and/or around the anterior ventral pouch which was more severe (graded as moderate) in two females exposed to 7.04 mg/m^3. Other associated treatment-related changes in males and females exposed to7.04 mg/m^3 and in one male exposed to 0.75 mg/m^3 were very slight or slight fibrosis within the lamina propria of the mucosa at the base of the epiglottis, very slight multifocal hemorrhages and the presence of small numbers of pigment-laden macrophages in the lamina propria of the mucosa at the base of the epiglottis and occasionally around the ventral pouch. A very slight or slight treatment-related focal hyperplasia of the lining epithelium of the ventral pouch was observed in some males and females exposed to 7.04 mg/m^3. There were no treatment related histopathological changes in the posterior larynx, trachea or lungs in males or females in any of the exposure groups. Females exposed to 7.04 mg/m^3 had treatment-related very slight or slight atrophy of the mesenteric adipose tissue which was interpreted to be secondary effect due to lower body weight and feed consumption as compared to those of the controls.

Under the conditions of this study, based on the histopathological effects on the larynx consistent with localized irritant effects of the test material at the portal of entry at 0.75 mg/m^3, the no-observed-effect- concentration (NOEC) of MBIT for Crl:CD(SD) rats of either sex, exposed via inhalation for six hours/day, five consecutive days/week for 13 weeks (65 exposures) was 0.19 mg/m^3.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

(Humans are not expected to be exposed to MBIT by other routes than the ones already tested: oral/dermal/inhalation). Therefore additional studies on other exposure routes are unlikely to yield any relevant new information and could be waived.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

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

14-day oral (drinking water) rat: based on the results of this range-finding oral (drinking water) study, the no-observed-effect level (NOEL) for oral (drinking water) administration of MBIT to Crl:CD(SD) rats for up to 14 consecutive days was 250 ppm. The no-observed-adverse-effect level (NOAEL) was 500 ppm.

90-day oral toxicity rats: toxicity of administration of m-BIT to Crl:CD(SD) rats via the drinking water for a minimum of 90 days was observed at the test substance concentration of 800 ppm (50 and 60 mg/kg/day for males and females, respectively), as evidenced by adversely decreased mean body weights, concurrent decreased food and water consumption, alterations in serum chemistry parameters and adverse microscopic findings in the stomach. Other than the microscopic findings in the stomach, the changes in body weights, food consumption and serum chemistry parameters were secondary to marked decreases in water consumption. Additional non-adverse effects included palatability-related lower mean water consumption noted at the test substance concentration of 50 ppm and non-adverse lower body weight gains and food and water consumption and alterations in serum chemistry parameters noted at the test substance concentration of 200 ppm. Based on results of this study, the **NOAEL** was considered to be 200 ppm (equivalent to 13-15 mg/kg/day).

90-day oral (dietary) dog study: based on the results of this study, MBIT, administered as dietary admixtures to Beagle dogs for a minimum of 90 days, was demonstrated to be poorly palatable at a concentration of 2000 ppm (59 and 67 mg/kg/day for males and females, respectively). Therefore, the no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL) for systemic toxicity was considered to be 750 ppm, corresponding to grand mean test substance exposures of 26 and 27 mg/kg/day for males and females, respectively. Due to the presence of hypertrophy of mucus-secreting gastric surface epithelial cells, the no-observed-adverse-effect level (NOAEL) for local stomach irritation was considered to be 250 ppm, corresponding to grand mean test substance exposures of 9 and 10 mg/kg/day for males and females, respectively.

Repeated dose toxicity - 4-week oral (dietary) dog: no overt toxicity was noted at the dietary concentrations used on this study (500, 1000, 2000, 2500, 3000, 4000 and 8000 ppm).

Subchronic inhalation toxicity study in rats – 90 days - the no-observed-effect-concentration (NOEC) of MBIT for Crl:CD(SD) rats of either sex, exposed via inhalation for six hours/day, five consecutive days/week for 13 weeks (65 exposures) was 0.19 mg/m^3.

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

No data available.

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

Not relevant for MBIT.

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

Not relevant for MBIT.

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

Data are available with MBIT by oral and dermal routes and by inhalation. In these studies, the only effects were observed at the site of dosing. Primary irritation was observed on the skin, in the digestive tract and in the respiratory tract. No evidence of systemic toxicity was observed in any study, given the lack of changes to the pathology of other tissues/organs. The effects reported do not appear severe enough to warrant classification. In conclusion, no classification for Specific Target Organ Toxicity (STOT) after repeated exposure (STOT RE) is required.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal The DS proposed to not classify MBIT for STOT RE, due to conclusive data not sufficient for classification. Repeated dose toxicity via oral route has been assessed both in rats and in dogs. In rats, 14 days range-finding study by administration of MBIT daily via the drinking water at the doses of 250, 500, 1 000 and 2 000 ppm (corresponding doses up to 83 mg/kg bw/d in males and up to 109 mg/kg bw/d in females) showed lower body weight gains and/or body weight losses, lower food and water consumption and clinical observations consistent with dehydration and poor nutrition (dermal atonia, thinness, decreased defecation and small faeces) mainly

at the two highest dose levels. All 2 000 ppm group males and females were euthanized on study day 11 due to body weight losses, low food and water consumption and poor general condition. There were no test substance related macroscopic findings at the scheduled necropsies or in the male rat (group 4, 1 000 ppm) that was found dead on study day 9.

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

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

Full 90-day study in dogs was conducted at the doses of 250, 750 and 2 000 ppm in diet (corresponding doses up to 59 mg/kg bw/d in males and up to 67 mg/kg bw/d in females). The study was performed in compliance with GLP, and assessed as reliable without restrictions by the DS. Clinical observations included thinness and/or dermal atonia, lower body weight gains (or body weight losses), and lower food consumption in 2 000 ppm group males and females. No test material related mortality occurred. In clinical chemistry, only effects observed were lower

absolute lymphocyte counts in the highest group females. Relative liver weights of the high dose group were higher than that of controls both in males and in females and absolute and relative adrenal weights were higher in high dose females than in controls. Only histopathological effects observed were, however, hypertrophy of the mucus-secreting cells of the surface epithelium in the stomach of 750 ppm and 2 000 ppm animals and thymic atrophy/involution in animals exposed to 2 000 ppm. Thymic effects were considered stress related and effects in stomach were considered to reflect irritant effect of MBIT on the gastric mucosa. None of the observed effects were considered relevant for classification.

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

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

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

In the males of the high dose group, mean absolute lung weights were significantly decreased (28.6 %), and mean relative testes weights were significantly increased. In females, mean absolute liver and ovary weights were

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

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

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

Comments received during public consultation

No comments were received regarding STOT RE.

Assessment and comparison with the classification criteria

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

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

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

In the case of MBIT, specific histopathological effects observed in both rats and dogs included local effects in forestomach or in gastric mucosa. In dogs, hypertrophy of the mucus-secreting cells of the surface epithelium in the stomach was seen at 750 ppm (26-27 mg/kg bw/d) and 2 000 ppm (59-67 mg/kg bw/d) in diet. In rats, there were minimal to mild inflammation and oedema and some focal erosions) in forestomach of some high dose (60 mg/kg bw/d) females. After inhalation, there were very slight or slight hyperplasia and hypertrophy of mucous cells, very slight, slight or moderate squamous metaplasia, very slight or slight subacute to chronic inflammation of the mucosa, very slight fibrosis within the

lamina propria of the mucosa, and very slight multifocal haemorrhages and the presence of small numbers of pigment-laden macrophages of the mucosa starting at 0.75 mg/m^3. These effects fit with the corrosive nature of the substance. Substance has been already classified as corrosive, and respiratory irritation has been also specifically addressed by STOT SE 3 classification. Thus, no STOT RE classification is warranted for these irritant effects.

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

In the rat 90 d oral study, total protein and globulin levels were slightly, but statistically significantly decreased and A/G ratio was increased in 200 and 800 ppm males. At these dose levels lower food consumption was observed in 800 ppm males but also in 200 ppm males at many time points. Urea nitrogen and phosphorus levels were slightly (but statistically significantly) increased at 800 ppm in females. This may be related to the reduced water consumption observed in these females. No clinical chemistry effects were observed in dogs. Since effects in clinical chemistry were only very mild, non-consistent across the studies and sexes and were likely to be related to the poor nutritional/hydration status of the animals, these effects are not considered relevant for classification.

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

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

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 32: Summary table of relevant in vitro mutagenicity studies.

Method	**Results**	**Remarks**	**Reference**
In vitro gene mutation study in bacteria Organism/cell type: *S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100 E. coli: WP2 *uvr*A Test guideline: OECD 471 Metabolic activated system: S-9 mix was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, 5 days prior to sacrifice. The S-9 mix contained: 10% S-9, 5 mM glucose-6-phosphate, 4 mM ß-nicotinamide-adenine dinucleotide phosphate, 8 mM magnesium chloride and 33 mM potassium chloride in a 100 mM phosphate buffer at 7.4 and was prepared immediately before use. Positive control: With metabolic activation: 2-aminoanthracene at 1.0 µg/plate for all 4 Salmonella strains and E. coli WP2 uvrA; Without metabolic activation: 2-nitrofluorene at 1.0 µg/plate for TA98, sodium azide at 1.0 µg/plate for TA100 and TA1535, 9-aminoacridine at 75 µg/plate for TA1537 and methyl methanesulfonate at 1000 µg/plate for E. coli WP2 uvrA. Overnight cultures of the bacteria strains were prepared by inoculating from the master plate or the appropriate frozen permanent stock into a vessel containing ~50 mL of culture medium. The tester strains were incubated at 37 ºC for approximately 12 hours before harvest. The bacteria were approximately 10^9 cells per ml when used in the assay.	MBIT is not mutagenic to *S. Typhimurium* or *E. Coli*	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.9% a.i.	Wagner V.O. and Klug M.L. (2006)

One-half (0.5) mL of S-9 or sham mix, 100 µl of tester strain and 50 µl of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45 ± 2 ºC. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 µl appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37 ± 2 ºC. Plates that were not counted immediately following the incubation period were stored at 2-8 ºC until colony counting could be conducted.			
In vitro mammalian chromosome aberration test Organism/cell type: <u>mammalian cell lines:</u> human peripheral blood lymphocytes (HPBL) obtained from a healthy, non-smoking 29 year old adult female human who had no recent history of radiotherapy, viral infection or the administration of drugs. Test guideline: OECD 473 Metabolic activation system: S-9 liver fraction from Aroclor 1254 induced male Sprague-Dawley rats. Positive control: Mitomycin C (MMC) at 0.3 and 0.6 µg/ml, non-activated Cyclophosphamide (CP) at 20 and 40 µg/ml, activated. Human peripheral blood lymphocytes were incubated at 37 ºC for 4 and 20 hours with MBIT treatment solutions in the –S-9 test and for 4 hours in the S-9-activated test. MBIT was dissolved in DMSO and dosed at 2.5, 5, and 10 µg/ml in the 4 hour incubation with and without S-9 metabolic activation and in the 20 hour incubation without S-9 metabolic activation.	MBIT is not mutagenic to human peripheral lymphocytes	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.9% a.i.	Gudi R. and Rao M. (2006)

In Vitro mammalian cell gene mutation test Organism/cell type: CHO-K_1 cells (Chinese hamster ovary cells) Test guideline: OECD 476 Metabolic activation system: Aroclor 1254-induced rat liver S9 Positive control: Ethyl methanesulfonate (EMS) at 0.2 µg/ml concentration as the positive control for the non-activated test system. Benzo(a)pyrene (B(a)P) at 4 µg/ml concentration as the positive control for the S-9 activated test system. CHO-K_1 cells were incubated at 37 ± 1ºC in a humidified atmosphere of 5 ± 1 % CO_2 in air for 18-24 hours prior to test initiation. Day 0 was the time of chemical treatment. Treatment flasks with CHO-K_1 cells were designated as non-activated or S-9 activated. 50 µl of dosing solution of MBIT, EMS positive control, B(a)P positive control, or DMSO vehicle alone were added to the treatment flasks per 25 cm^2 surface area. Duplicate flasks of cells were exposed to at least five concentrations of MBIT for 5 hours at 37 ± 1ºC. After the treatment period, all media were aspirated, the cells washed with Calcium and Magnesium free Hanks'balanced salt solution and cultured in F12FBS5-Hx for an additional 18-24 hours at 37 ± 1ºC. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period. For evaluation of cytotoxicity, the replicates from each treatment were subcultured at a density of 100 cells/60 mm dish. After 7-10 days of incubation the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted and cloning efficiency determined.	MBIT is not mutagenic in the CHO/HGPRT mutation assay	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Clarke J.J. (2009)

4.9.1.2 In vivo data

Table 33: Summary table of relevant in vivo mutagenicity studies.

Method	Results	Remarks	Reference
Genotoxicity In Vivo micronucleus assay Species: Mouse Strain: ICR Test guideline: OECD 474	MBIT is not mutagenic in the mouse micronucleus assay	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Krsmanovic L. and Huston T. (2009)

4.9.2 Human information

Not available.

4.9.3 Other relevant information

None.

4.9.4 Summary and discussion of mutagenicity

***In vitro* gene mutation study in bacteria:**

In the initial mutation assay, no positive mutagenic responses were observed. No precipitate was observed. Toxicity was observed beginning at 50, 150 or 500 µg a.i./plate. In the confirmatory assay, no positive mutagenic responses were observed. No precipitate was observed but toxicity was observed beginning at 150 µg a.i./plate.

Table 34: Table for *Salmonella typhimurium* and *Escherichia coli* WP2*uvr*AGene Mutation

Assay – initial assay

Average Revertants Per Plate ± Standard Deviation						
Dose [µg/plate]	S-9	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Vehicle, DMSO	-	15 ± 5	118 ± 7	15 ± 1	8 ± 5	11 ± 1
1.5	-	10 ± 4	107 ± 4	15 ± 5	9 ± 0	16 ± 4
5.0	-	16 ± 1	90 ± 11	15 ± 2	8 ± 0	15 ± 6
15	-	14 ± 1	100 ± 11	13 ± 1	8 ± 1	15 ± 0
50	-	13 ± 6	124 ± 14	12 ± 4	10 ± 1	18 ± 8
150	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
500	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1500	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5000	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	-	111 ± 20	579 ± 15	223 ± 6	567 ± 73	117 ± 19
Vehicle, DMSO	+	28 ± 4	124 ± 0	17 ± 1	9 ± 1	16 ± 3
1.5	+	24 ± 5	109 ± 10	15 ± 1	6 ± 1	20 ± 7
5.0	+	21 ± 7	131 ± 4	14 ± 1	7 ± 1	15 ± 41
15	+	25 ± 0	111 ± 13	14 ± 4	10 ± 01	17 ± 1
50	+	14 ± 3	148 ± 10	11 ± 1	4 ± 1	14 ± 0
150	+	0 ± 0	0 ± 0	0 ± 0	6 ± 1	19 ± 4
500	+	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1500	+	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5000	+	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	+	277 ± 78	518 ± 6	79 ± 18	44 ± 6	195 ± 64

50 µl plating aliquot

Table 35: Table for *Salmonella typhimurium* and *Escherichia coli* WP2*uvr*AGene Mutation

Assay – confirmatory assay

Average Revertants Per Plate ± Standard Deviation						
Dose µg/plate]	S-9	TA98	TA100	TA1535	TA1537	WP2*uvr*A
Vehicle	-	13 ± 5	122 ± 8	17 ± 1	7 ± 3	12 ± 2
1.5	-	18 ± 6	116 ± 24	17 ± 1	8 ± 3	15 ± 2
5.0	-	12 ± 4	122 ± 10	17 ± 4	7 ± 3	12 ± 3
15	-	19 ± 5	125 ± 21	19 ± 4	7 ± 2	14 ± 4
50	-	17 ± 5	130 ± 20	19 ± 10	4 ± 1	13 ± 5
150	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
500	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	-	142 ± 15	638 ± 121	333 ± 40	1067 ± 36	119 ± 7
Vehicle	+	20 ± 2	115 ± 9	16 ± 7	6 ± 3	18 ± 5
1.5	+	21 ± 5	117 ± 4	15 ± 2	--*	--*
5.0	+	19 ± 5	116 ± 7	10 ± 3	7 ± 1	17 ± 7
15	+	25 ± 7	125 ± 20	15 ± 9	7 ± 6	17 ± 3
50	+	20 ± 4	122 ± 12	15 ± 6	8 ± 0	14 ± 4
150	+	6 ± 11	0 ± 0	7 ± 2	3 ± 3	4 ± 7
500	+	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1500	+	--*	--*	--*	0 ± 0	0 ± 0
Positive control	+	306 ± 48	619 ± 11	102 ± 39	88 ± 6	239 ± 17

50 µl plating aliquot

* not tested

In vitro mammalian chromosome aberration test

The percentage of cells with structural or numerical aberrations in the MBIT-treated groups was not significantly increased above that of the DMSO solvent control at any dose level ($p>0.05$ Fisher's exact test). The positive and solvent control groups fulfilled the requirements for a valid test.

Table 36: *In-Vitro* Chromosomal Analysis, Definitive

Without activation, 4 hour treatment, 20 hour harvest		**DMSO control**	**MMC 0.6 µg/ml**	**MBIT 2.5 µg/ml**	**MBIT 5 µg/ml**	**MBIT 10µg/ml**
Average aberrations per cell		0.000	0.150	0.000	0.000	0.010
% Aberrant cells	Numerical	0	0	0	0	0
	Structural	0	15	0	0	1
Total number of Chromatid structural aberrations	Gaps	0	0	0	0	0
	Breaks (Br)	0	3	0	0	1
	Exchanges (Ex)	0	2-3	0	0	0
Total number of Chromosome structural aberrations	Breaks (Br)	0	1-2	0	0	0
	Dicentrics (Dic)	0	0-1	0	0	0
	Ring	0	0	0	0	0
Severely damaged cells		0	0	0	0	0
Mitotic index (%)		13.4	11.7	12.3	12.0	6.2
*** p ≤ 0.05; ** p ≤ 0.01; using Fisher's exact test**						

Table 37: *In-Vitro* Chromosomal Analysis, Definitive

Without activation, 20 hour treatment, 20 hour harvest		**DMSO control**	**MMC 0.6 µg/ml**	**MBIT 2.5 µg/ml**	**MBIT 5 µg/ml**	**MBIT 10µg/ml**
Average aberrations per cell		0.000	0.180	0.000	0.000	0.005
% Aberrant cells	Numerical	0	0	0	0	0
	Structural	0	17	0	0	0-1
Total number of Chromatid structural aberrations	Gaps	0	0	0	0	0
	Breaks (Br)	0	0-1	0	0	0-1
	Exchanges (Ex)	0	0	0	0	0
Total number of Chromosome structural aberrations	Breaks (Br)	0	0	0	0	0
	Dicentrics (Dic)	0	0	0	0	0
	Ring	0	0	0	0	0
Severely damaged cells		0	0	0	0	0
Mitotic index (%)		13.3	11.4	13.0	12.2	5.6
*** p ≤ 0.05; ** p ≤ 0.01; using Fisher's exact test**						

Table 38: *In-Vitro* Chromosomal Analysis, Definitive

With activation, 4 hour treatment, 20 hour harvest		**DMSO control**	**MMC 0.6 µg/ml**	**MBIT 2.5 µg/ml**	**MBIT 5 µg/ml**	**MBIT 10µg/ml**
Average aberrations per cell		0.000	0.150	0.000	0.000	0.000
% Aberrant cells	Numerical	0	0	0	0	0
	Structural	0	14	0	0	0

Total number of Chromatid structural aberrations	Gaps	0	0	0	0	0
	Breaks (Br)	0	3-5	0	0	0
	Exchanges (Ex)	0	0-3	0	0	0
Total number of Chromosome structural aberrations	Breaks (Br)	0	1-3	0	0	0
	Dicentrics (Dic)	0	0	0	0	0
	Ring	0	0	0	0	0
Severely damaged cells		0	0	0	0	0
Mitotic index (%)		13.5	11.4	12.1	11.9	6.4
*** $p \leq 0.05$; ** $p \leq 0.01$; using Fisher's exact test**						

In Vitro mammalian cell gene mutation test

In the initial mutagenesis assay, no positive responses, i.e., treated cultures with mutant frequencies > 40 mutants per 10^6 clonable cells, were observed. No visible precipitate was observed in MBIT treatment medium at any concentration. Toxicity was observed at concentrations of ≥ 5.0 µg/ml MBIT without S9 activation and ≥ 10 µg/ml MBIT with S9 activation.

In the confirmatory mutagenesis assay, no positive responses were observed. No visible precipitate was observed in MBIT treatment medium at any concentration. Toxicity was observed at concentrations of ≥ 4.0 µg/ml MBIT without S9 activation and ≥ 8.0 µg/ml MBIT with S9 activation.

Table 39: Table for gene mutation assay - Concurrent Cytotoxicity Test (initial assay, definitive)

Treatment (µg/ml MBIT)	**Non-activated**		**Treatment (µg/ml MBIT)**	**S-9 Activated**	
	Cloning Efficiency (%)			**Cloning Efficiency (%)**	
	Total	**Relative**		**Total**	**Relative**
Solvent (DMSO)	74	100	Solvent (DMSO)	71	100
EMS (0.2 µl/ml)	39	52	B(a)P (4 µg/ml)	30	42
1	65	89	1	65	91
2.5	38	52	5	55	77
5	9	12	10	27	38
7.5	4	5	15	35	49
10	0	0	25	0	0
Cloning efficiency = total colonies counted / number of dishes x 100 cells/dish Relative cloning efficiency = (cloning efficiency treatment group/cloning efficiency solvent group) x 100					

Table 40: Table for gene mutation assay – initial assay (definitive)

Non-activated CHO/HGPRT Initial Assay

Cloning Efficiency Plates		**Cloning Efficiency**	**Selection (Mutation) Plates**	**Mutants / 10^6 Clonable Cells**
Treatment (µg/ml MBIT)	**Average Colonies**		**Average Colonies**	
Solvent (DMSO)	51.6	0.52	0	0
EMS (0.2 µl/ml)	46.7	0.47	20.1	215.5
1	50.7	0.51	0.8	7.7
2.5	60.8	0.61	0	0
5	40.0	0.40	0	0
7.5		***		***
10		***		***
20		***		***
*** Culture not cloned due to excessive toxicity Cloning efficiency = average colonies / 100 cells per dish Mutants/10^6 clonable cells = 9average mutant colonies / cloning efficiency X 2 x 10^5 cells) x 10^6				

Activated (+S9) CHO/HGPRT Initial Assay

Cloning Efficiency Plates		**Cloning Efficiency**	**Selection (Mutation) Plates**	**Mutants / 10^6 Clonable Cells**
Treatment (µg/ml MBIT)	**Average Colonies**		**Average Colonies**	
Solvent (DMSO)	62.2	0.62	0.2	1.6
B(a)P (4 µl/ml)	52.0	0.52	12.8	123.1
1	70.8	0.71	0.2	1.4
5	53.0	0.53	0.7	6.6
10	58.2	0.58	0.3	2.6
15	73.5	0.74	0	0
25		***		***
50		***		***
*** Culture not cloned due to excessive toxicity Cloning efficiency = average colonies / 100 cells per dish Mutants/10^6 clonable cells = 9average mutant colonies / cloning efficiency X 2 x 10^5 cells) x 10^6				

Table 41: Table for gene mutation assay - Concurrent Cytotoxicity Test (confirmatory assay)

Treatment (µg/ml m,BIT)	**Non-activated**		**Treatment (µg/ml MBIT)**	**S-9 Activated**	
	Cloning Efficiency (%)			**Cloning Efficiency (%)**	
	Total	**Relative**		**Total**	**Relative**
Solvent (DMSO)	58	100	Solvent (DMSO)	65	100
EMS (0.2 µl/ml)	44	76	B(a)P (4 µg/ml)	24	37
0.5	53	91	6	53	81
1	41	71	8	23	36
2	32	55	10	13	20
3	35	59	15	7	11
4	13	22	17.5	11	16
5	16	27	20		***
*** Culture not cloned due to excessive toxicity Cloning efficiency = total colonies counted / number of dishes x 100 cells/dish Relative cloning efficiency = (cloning efficiency treatment group/cloning efficiency solvent group) x 100					

Table 42: Table for gene mutation assay – confirmatory assay

Non-activated CHO/HGPRT Initial Assay

Cloning Efficiency Plates		**Cloning Efficiency**	**Selection (Mutation) Plates**	**Mutants / 10^6 Clonable Cells**
Treatment (µg/ml MBIT)	**Average Colonies**		**Average Colonies**	
Solvent (DMSO)	84.2	0.84	0	0
EMS (0.2 µl/ml)	61.7	0.62	39.5	320.3
0.5	78.8	0.79	0	0
1	79.5	0.80	0.1	0.6
2	78.5	0.79	0.4	2.5
3	70.8	0.71	0.9	6.4
4	60.3	0.60	0	0
5	57.3	0.57	0	0
Cloning efficiency = average colonies / 100 cells per dish Mutants/10^6 clonable cells = 9average mutant colonies / cloning efficiency X 2 x 10^5 cells) x 10^6				

Activated (+S9) CHO/HGPRT Initial Assay

Cloning Efficiency Plates		**Cloning Efficiency**	**Selection (Mutation) Plates**	**Mutants / 10^6 Clonable Cells**
Treatment (µg/ml MBIT)	**Average Colonies**		**Average Colonies**	
Solvent (DMSO)	70.5	0.71	0	0
B(a)P (4 µl/ml)	82.2	0.82	24.0	146.0
6	52.7	0.53	0	0
8	76.3	0.76	0	0
10	44.5	0.45	0	0
15	52.5	0.53	0	0
17.5	53.0	0.53	0	0
20	***	***	0	0
*** Culture not cloned due to excessive toxicity Cloning efficiency = average colonies / 100 cells per dish Mutants/10^6 clonable cells = 9average mutant colonies / cloning efficiency X 2 x 10^5 cells) x 10^6				

Genotoxicity In Vivo micronucleus assay

Based on the results of the range-finding study, the high dose for the definitive study was set at 200 mg MBIT/kg body weight which was estimated to be the maximum tolerated dose. At the time of euthanasia (24 or 48 hours), femoral bone marrow was collected and bone marrow smears (slides) were prepared and stained with May-Gruenwald-Giemsa stain.

Table 43: Table for Micronucleus Test *In Vivo*

State mean ± standard deviation state individual numbers for critical findings		**control group (0.5% methylcellulose /0.1% Tween 80 in purified water)**		**low dose (50 mg/kg)**	**mid dose (100 mg/kg)**	**high dose (200 mg/kg)**	
Number of cells evaluated/mouse		2000		2000	2000	2000	
Sampling time (h)		24 h	48 h	24 h	24 h	24 h	48 h
Ratio of erythrocytes	MPCE/1000 PCE, males	0.2±0.27	0.0±0.00	0.3±0.45	0.2±0.27	0.1±0.22	0.0±0.00
Ratio of erythrocytes	MPCE/1000 PCE, females	0.5±0.35	0.3±0.45	0.2±0.27	0.1±0.22	0.2±0.27	0.1±0.22

Ratio of erythrocytes	PCE/total erythrocytes (PCE/EC ratio), males	0.495±0.05	0.387±0.04	0.426±0.08	0.465±0.04	0.352±0.09	0.406±0.03
Ratio of erythrocytes	PCE/total erythrocytes (PCE/EC ratio), females	0.415±0.10	0.486±0.07	0.458±0.06	0.446±0.04	0.412±0.04	0.523±0.06

4.9.5 Comparison with criteria

***In vitro* gene mutation study in bacteria:**

MBIT did not induce point mutations in *S. Typhimurium* tester strains TA 1535, TA 1537, TA 98, TA 100 and E. Coli strain WP2 *uvr*A, both with and without metabolic activation.

***In vitro* mammalian chromosome aberration test**

MBIT was negative (not mutagenic) for the induction of structural and numerical chromosome aberrations in the *in vitro* mammalian chromosome aberration test using human peripheral blood lymphocytes in both the non-activated and the S-9 activated test systems.

***In Vitro* mammalian cell gene mutation test**

MBIT was not mutagenic in the CHO/HGPRT mutation assay.

Genotoxicity In Vivo micronucleus assay

Mortality was observed in mice dosed with 200 mg MBIT/kg and 300 mg MBIT/kg in the range-finding study and in mice dosed with 200 mg MBIT/kg in the definitive study. Final mean concentrations and %RSD for 2.5, 5 and 10 mg/ml were 2.5, 5.23 and 9.82 mg/ml with 6.12, 1.14 and 0.98% RSD, respectively. Under the conditions described in this report, a single oral administration of MBIT at doses up to and including a dose of 200 mg MBIT/kg body weight did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of male or female ICR mice. Therefore, MBIT was concluded to be negative in the mouse micronucleus assay.

4.9.6 Conclusions on classification and labelling

MBIT based on the following studies is not classified, according to CLP, as mutagenic:

- *In vitro* gene mutation study in bacteria,

- *In vitro* mammalian chromosome aberration test,
- *In Vitro* mammalian cell gene mutation test,
- Genotoxicity In Vivo micronucleus assay.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

Table. Summary of the mutagenicity studies

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

Comments received during public consultation

No comments were received concerning germ cell mutagenicity.

Assessment and comparison with the classification criteria

Substances are classified for mutagenicity in Cat. 1, if they are known to induce heritable mutations or are regarded as if they induce heritable mutations in the germ cells of humans. Cat. 2 applies to substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the

germ cells of humans, based on positive evidence obtained from *in vivo* experiments in mammals and/or in some cases from *in vitro* experiments.

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

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

No data available.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

See Point 4.10.4.

4.10.4 Summary and discussion of carcinogenicity

MBIT is one of a number of isothiazolinone molecules which have application as broad spectrum biocides in a wide range of industrial and consumer products, the vast majority of which have been tested for toxicity.

There are no data, from studies conducted according to current guidelines, addressing the chronic and carcinogenicity endpoints for MBIT. However, the compound has been tested for toxicity in a number of assays with repeat dose protocols, including 90 day studies in the rat and in the dog and a range of studies focusing on reproductive

end-points. In addition there is genotoxicity data from a number of *in-vivo* and *in-vitro* test systems and its ADME has been studied.

A common feature of the repeat dose study with MBIT is that, irrespective of the species or the route of administration, the major toxicity observed is irritation/corrosion at the site of primary contact (subsequently any clinical signs of toxicity or mortality are secondary to these irritant effects). In none of the studies were there any histopathological effects in any tissues distant from the site of dosing (i.e., no end-organ toxicity) and in no case was there evidence suggestive of a potential endocrine mechanism of carcinogenesis.

It would be reasonable to conclude that chronic exposure would also demonstrate site of contact toxicity in the form of irritation/corrosion.

In vitro and *in-vivo* genotoxicty studies on MBIT were negative thus arguing against a potential genotoxic mechanism of carcinogenesis.

Oral ADME study indicates that MBIT, as with other isothiazolones, was extensively metabolized following a single or multiple doses to the rat. Unchanged MBIT was not found in urine or feces. The metabolite profiles of male and female rat urine from the multiple oral dose group (Group 6) were similar to those of the single dose group. Overall, the findings indicate that MBIT does not bioaccumulate in rat tissues. Most of the dosed radioactivity was recovered within 24 hr post-dose for all groups. Total mean recoveries from all mass balance groups were all greater than 97%. There was no gender difference in the excretion pattern.

There is no evidence that either MBIT or its metabolites bioaccumulate following repeated oral exposure, this is consistent with the lack of systemic toxicity observed in multidose studies (A6.4.1.a/01 90 day oral rat and A6.4.1.b/01 90 day oral dog).

Given the lack of significant end-organ toxicity, genotoxic potential and endocrine activity, it may be concluded that 2-Methyl-1,2-Benzisothiazolin-3-one is unlikely to demonstrate a carcinogenic potential.

The probable lack of carcinogenicity of MBIT can be supported by consideration of two other isothiazolinones, CMIT/MIT (which is a mixture of 5-chloro-2-methyl-2H-isothiazolin-3-one and 2-methyl-2H-isothiazolin-3-one, in the ratio of 3:1) and OIT (2-n-octyl-4-isothiazolin-3-one). These have also been extensively tested in repeat dose studies which include three chronic studies.

Thus CMIT/MIT and OIT have demonstrated, like MBIT, point of contact toxicity [in the form of irritation and/or corrosion] in a range of repeat dose studies, including those addressing reproduction endpoints. In common with MBIT, CMIT/MIT and OIT have shown no significant systemic toxicity and provide no evidence to suggest a possible endocrine mechanism of carcinogenicity. CMIT/MIT and OIT, like MBIT, show some genotoxicity *in-vitro* but are not genotoxic *in-vivo*. Importantly, neither of the carcinogenicity studies conducted on CMIT/MIT [one by the oral (drinking water) route and one by skin "painting"] or OIT [one by oral (dietary) exposure] indicate a carcinogenic potential.

Metabolism of CMIT/MIT and OIT is initiated through a nucleophilic attack on the sulphur-nitrogen bond (an electrophilic center) to open the isothiazolinone ring.

Subsequently, CMIT/MIT and OIT are metabolized to malonamic acid type derivatives- n-methyl-malonamic acid and n-octyl-malonamic acid (and further derivatives), respectively.

Opening of the isothiazolinone ring significantly reduces biological activity.

Studies with N-methyl malonamic acid indicate that the compound is not mutagenic and not a sensitizer, as compared to the parent compound (CMIT/MIT). Similarly, n-octyl-malonamic acid is not mutagenic and not a sensitizer, as compared to the parent compound (OIT).

From a comparison of the toxicological data on these three materials (MBIT, CMIT/MIT and OIT) it would seem that there is a common theme: they demonstrate rapid metabolism (detoxification pathways), toxicity is at the site of dosing, with no discernable systemic effects and neither material suggests an endocrine or genotoxic mechanism for carcinogenicity. Based on the results of these studies, it can be concluded that the conduct of chronic/carcinogenicity studies with MBIT will add little or nothing to the human health risk assessment of MBIT and could be waived.

4.10.5 Comparison with criteria

There are no relevant data to compare with criteria (No experimental studies were performed to assess the carcinogenicity potential of substance).

4.10.6 Conclusions on classification and labelling

Classification and labelling is not required (justification – see Point 4.10.4).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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

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

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

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

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

Comments received during public consultation

No comment were received during public consultation regarding carcinogenicity.

Assessment and comparison with the classification criteria

A substance is classified in Cat. 1A or 1B for carcinogenicity on the basis of

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

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Table 44: Summary of relevant toxicity for reproduction study – effects on fertility.

Method	Results	Remarks	Reference
Multigeneration Reproduction Toxicity Study – Rat Oral (drinking water) Species: Rats Sex: Males and females Strain: Crl:CD(SD) Test guideline: OECD 416 GLP: yes Number of animals per group F0 generation: 30/sex/group F1 generation: 30/sex/group Mating: Vaginal lavages were performed daily for determination of estrous cycles beginning 21 days prior to pairing. Duration of mating: Approximately 2 weeks mating period for F0 and F1. Duration of exposure in general P, F1, F2 males, females: The F0 and F1 males continued to be exposed to the test substance throughout mating, and through the day of euthanasia. The F0 and F1 females continued to be exposed to the test substance throughout mating, gestation and lactation, and through the day of euthanasia.	NOAEL = 200 ppm for parental systemic toxicity and neonatal toxicity NOAEL = 400 ppm for parental reproductive toxicity LO(A)EL Parent males (F0) LOAEL = 800/400 ppm for parental systemic toxicity (20 to 50 mg/kg/day) Parent females (F0) LOAEL = 800/400 ppm for parental systemic toxicity (59 to 113 mg/kg/day) F1 males LOAEL = 400 ppm for parental systemic toxicity (23 to 39 mg/kg/day) LOAEL = 800/400 ppm for neonatal toxicity F1 females LOAEL = 400 ppm for parental systemic toxicity (41 to 83 mg/kg/day) LOAEL = 800/400 ppm for neonatal toxicity F2 males LOAEL = 400 ppm for	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Stump D.G. (2009a)

F0 males and females were exposed for 134 - 138 consecutive days and F1 males and females were directly exposed for 138 - 151 consecutive days. Concentration: the initial exposure levels were 50, 200 and 800 parts per million (ppm) for the F0 generation. Due to excessive toxicity (marked reductions in mean F0 and F1 pup body weights) noted in the 800 ppm group, this exposure level was reduced to 400 ppm (F0 male study week 16 or F0 female lactation days/F1 pup postnatal days [PND] 15 20) and maintained at this exposure level throughout the remainder of the F0 generation and for the entire F1 generation. Clinical signs: all animals were observed twice daily for appearance and behavior. Clinical observations were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Body weight: body weights were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Food/water consumption: water and food consumption were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Sperm parameters: Spermatogenic endpoints (sperm motility [including progressive motility], morphology and numbers) were recorded for all F0 and F1 males. Microscopic evaluations were made on the following tissues for F0 and F1 males: epididymis, testes, and seminal vesicles. The following organs were weighed: epididymis (total and cauda), prostate gland, testes and seminal	neonatal toxicity F2 females LOAEL = 400 ppm for neonatal toxicity NO(A)EL Parent males (F0) NOAEL = 200 ppm for parental systemic toxicity (10 to 14 mg/kg/day) Parent females (F0) NOAEL = 200 ppm for parental systemic toxicity (19 to 44 mg/kg/day) F1 males NOAEL = 200 ppm for parental systemic toxicity (11 to 18 mg/kg/day) NOAEL = 400 ppm for parental reproductive toxicity (23 to 39 mg/kg/day) NOAEL = 200 ppm for neonatal toxicity F1 females NOAEL = 200 ppm for parental systemic toxicity (21 to 45 mg/kg/day) NOAEL = 400 ppm for parental reproductive toxicity (41 to-83 mg/kg/day) NOAEL = 200 ppm for neonatal toxicity F2 males NOAEL = 200 ppm for neonatal toxicity F2 females NOAEL = 200 ppm for neonatal toxicity Parent males: One F0 male each in the 200 and 800/400 ppm groups was euthanized in extremis or found dead. However, the moribundity of the single male in the 800/400 ppm group was attributed to a mechanical injury and not to test substance exposure. Additionally, the mortality of the male in the 200 ppm group was not attributed to test substance exposure due to the absence of an exposure-related trend. All other F0 and all F1		

vesicles with coagulating glands. Clinical observations (offspring), body weights and sexes for F1 and F2 pups were recorded at appropriate intervals. Each litter was examined twice daily for survival, and all deaths were recorded. A daily record of litter size was maintained. Intact offspring dying from PND 0 to 4 were necropsied. A detailed gross necropsy was performed on any pup dying or euthanized in extremis after PND 4 and prior to weaning. Tissues were preserved for possible future histopathological examination only as deemed necessary by the gross findings. Litters were examined daily for any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1, 4, 7, 14 and 21; all F1 pups selected to constitute the F1 generation also received a detailed physical examination on PND 28. Any abnormalities in nursing behavior were recorded. Pups were individually weighed on PND 1, 4, 7, 14 and 21; all F1 pups selected to constitute the F1 generation were also weighed on PND 28. Pups were individually sexed on PND 0, 4, 14 and 21. Each male pup was observed for balanopreputial separation beginning on PND 35. The age at which balanopreputial separation was first observed was recorded for each pup. Examination of the pups continued daily until balanopreputial separation was present. Body weights were recorded at the age of attainment of this landmark. Each female pup was observed for vaginal perforation beginning on PND 25. The age at which the vaginal lumen was first observed to open was recorded for each pup. Examination of the females was continued daily until vaginal patency was present. Body weights were recorded at the age of	parental animals survived to the scheduled necropsies. Test substance-related lower mean body weight gains were noted in the 800/400 ppm group F0 males generally throughout exposure at 800 ppm. Following the reduction in the exposure level from 800 ppm to 400 ppm on study week 16, mean body weight gains for these F0 males were slightly higher than the control group values. Mean water consumption for F0 males in the 50, 200 and 800/400 ppm groups was generally reduced in an exposure-related manner in the entire generation. Parent females: One F0 female each in the control and 200 ppm groups was euthanized in extremis or found dead. Additionally, the mortality of the female in the 200 ppm group was not attributed to test substance exposure due to the absence of an exposure-related trend. All other F0 and all F1 parental animals survived to the scheduled necropsies. F0 female body weight gain in the 800/400 ppm group was only affected during the first week of exposure. Mean body weights, body weight gains and food consumption of the F0 females in the 200 ppm group were unaffected by test substance exposure throughout the pre-mating, gestation and lactation periods. Mean water consumption for F0 females in the 50, 200 and 800/400 ppm groups was generally reduced in an exposure-related manner throughout the pre-mating, gestation and lactation periods. F1 males: mean water consumption for F1 males in the 50, 200 and 400 ppm groups was generally reduced in an exposure-related manner throughout the entire generation. Mean water consumption for F1 pups in the 400 ppm group was reduced and water consumption in the 50 and 200 ppm groups		

attainment of this landmark. Organ weights P and F1: Selected organs were weighed for 1 pup/sex/litter from both F1 and F2 pups that were necropsied on PND 21. The following organs were weighed from all F0 and F1 parental animals at the scheduled necropsies: adrenal glands, brain, epididymides (total and cauda), kidneys, liver, ovaries, pituitary, prostate, seminal vesicles with coagulating glands, spleen, testes, thyroid, thymus, and uterus with oviducts and cervix. Histopathology P and F1: designated tissues from all F0 and F1 parental animals in the control and high-exposure groups and the kidneys from all F0 and F1 parental animals in all groups were examined microscopically. Additionally, the reproductive organs of all animals suspected of reduced fertility in the low- and mid-exposure groups were examined microscopically. Histopathology F1 not selected for mating, F2: Gross necropsies with emphasis on developmental morphology and organs of the reproductive system were performed on non-selected F1 and F2 pups euthanized on PND 21. The selected F1 and F2 organs (brain, spleen and thymus) were collected from 1 pup/sex/litter that survived to the scheduled termination on PND 21. These tissues and all gross lesions from F1 and F2 weanlings were preserved in 10% neutral buffered formalin for possible future histopathologic examination; all other tissues and the carcasses were discarded.	was unaffected by test substance exposure when housed by litter during PND 28-35. Slightly lower F1 pup body weights were noted on PND 1 in the 800/400 ppm group compared to the control group and F1 male pup body weight gains in this group were lower throughout the pre-weaning period compared to the control group. As a result, mean F1 male pup body weights in the 800/400 ppm group were 11.3% to 40.9% lower, respectively, during PND 4 28, and a delay in the mean age and a lower mean body weight on the day of attainment of balanopreputial separation was noted for F1 male pups in this group. Mean body weights and body weight gains for F1 pups in the 50 ppm and 200 ppm groups were unaffected by test substance exposure. Test substance-related clinical findings in the F1 pups in the 800/400 ppm group included uneven hair growth, striped hair growth and unkempt appearance; these findings were noted on PND 14 and/or 21. Pale body was also noted for 8 pups in the 800/400 ppm group. No clinical findings noted for F1 pups in the 50 and 200 ppm groups were attributed to test substance exposure. F1 females: Mean body weight gain for F1 females in the 200 ppm group was lower on study week 19-20, and as a result of the initial reduction in mean body weight gain, mean F1 female body weights in the 200 ppm group were generally reduced throughout the study. Mean food consumption for F1 females in the 200 ppm group was generally similar to the control group throughout the pre-mating, gestation and lactation periods. Mean water consumption for F1 females in the 50, 200 and 400 ppm groups was generally reduced in an		

	exposure-related manner throughout the pre-mating, gestation and lactation periods. Mean water consumption for F1 pups in the 400 ppm group was reduced and water consumption in the 50 and 200 ppm groups was unaffected by test substance exposure when housed by litter during PND 28-35. Slightly lower F1 female pup body weights were noted on PND 1 in the 800/400 ppm group compared to the control group and F1 female pup body weight gains in this group were lower throughout the pre-weaning period compared to the control group. As a result, mean F1 female pup body weights in the 800/400 ppm group were 9.7% to 40.2% lower, during PND 4 28, and a delay in the mean age and a lower mean body weight on the day of attainment of vaginal patency were noted for F1 female pups, in this group. Mean body weights and body weight gains for F1 pups in the 50 ppm and 200 ppm groups were unaffected by test substance exposure. Test substance-related clinical findings in the F1 pups in the 800/400 ppm group included uneven hair growth, striped hair growth and unkempt appearance; these findings were noted on PND 14 and/or 21. Pale body was also noted for 8 pups in the 800/400 ppm group. No clinical findings noted for F1 pups in the 50 and 200 ppm groups were attributed to test substance exposure. F2 males: Test substance-related lower mean F2 male pup body weight gains were noted during PND 7-14 and/or 14-21 in the 200 and 400 ppm groups. As a result, mean F2 pup body weights in the 200 and 400 ppm groups were 8.4% to 9.0% lower on PND 21 compared to the control group.		

	Mean body weights and body weight gains for F2 pups in the 50 ppm group were unaffected by test substance exposure. No clinical findings noted for F2 pups in the 50, 200 and 400 ppm groups were attributed to test substance exposure. F2 females: Test substance-related lower mean F2 female pup body weight gains were noted during PND 7-14 and/or 14-21 in the 200 and 400 ppm groups. As a result, mean F2 pup body weights in the 200 and 400 ppm groups were 16.9% to 18.0% lower on PND 21 compared to the control group. Mean body weights and body weight gains for F2 pups in the 50 ppm group were unaffected by test substance exposure. No clinical findings noted for F2 pups in the 50, 200 and 400 ppm groups were attributed to test substance exposure.		

4.11.1.2 Human information

No relevant information is available.

4.11.2 Developmental toxicity

Table 45: Summary of relevant toxicity for reproduction study – developmental toxicity.

Method	Results	Remarks	Reference
Teratogenicity Study – Rat Oral (gavage) Species: Rats Strain: Crl:CD(SD) Sex: Sexually mature, virgin females and males of same strain and source. Number of animals per group: 25 females per group Test guideline: OECD 414 GLP: yes Duration of exposure: Rat: day 6-19 post mating	NOAEL = 5 mg/kg/day for maternal toxicity and embryo-fetal development LOAEL = 18 mg/kg/day for maternal toxicity and embryo-fetal development Maternal toxic Effects: In the 18 mg/kg/day group, 3/25 females were euthanized in extremis on gestation day 9 or 11 due to body weight losses, reduced food consumption, and poor clinical condition. Test	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Stump D.G. (2009b)

Concentration: Dosage levels were 2, 5, and 18 mg/kg/day. Dosage levels were determined from the results of the dose range-finding prenatal developmental toxicity study in rats. In the dose range-finding study, mortality and indications of stomach irritation were observed at dosage levels of 75, 150, and 300 mg/kg/day. Furthermore, moribundity, rales, gasping, and body weight losses and/or lower body weight gains were noted at ≥ 20 mg/kg/day and reduced food consumption was noted at ≥ 30 mg/kg/day. In addition, effects on intrauterine survival were observed in the 30 mg/kg/day group. Based on these results, dosage levels of 2, 5, and 18 mg/kg/day were selected for this developmental toxicity study. Examinations Body weight: Yes, Individual maternal body weights were recorded on gestation days 0 and 6-20 (daily). Food consumption: Yes, Individual food consumption was recorded on gestation days 0 and 6-20 (daily). Clinical signs: Yes, All animals were observed twice daily for mortality and moribundity. Animals were also observed for signs of toxicity approximately 1 hour following dose administration. Examination of uterine content Gravid uterine weights were recorded Number of corpora lutea were recorded Number of implantations were recorded Early and late resorptions were recorded Examination of foetuses General Litter Size, Number of litters/group, Number of dead Foetuses, Foetal Weight, Sex Ratio Skeletal: Yes, All fetal carcasses were prepared, stained and examined for skeletal morphology. Heads from one-half of the fetuses in each group examined by a mid	substance-related clinical observations noted for females euthanized in extremis or at the scheduled termination in the 18 mg/kg/day group included rales, gasping, limbs and/or body cool to touch, red material on the nose, mouth, and/or forelimbs, and/or yellow material on the anogenital or urogenital areas at the daily examinations and/or approximately 1 hour following dose administration generally beginning with the initiation of treatment and continuing until euthanasia. These findings were most likely associated with the locally irritating nature of the test substance and not a result of direct systemic toxicity. There were no test substance related clinical findings noted in the 2 and 5 mg/kg/day groups. Teratogenic/embryo-toxic effects: Mean fetal weights in the 18 mg/kg/day group were 7.9% (male) and 10.5% (female) lower than the concurrent control group values and were considered to be test substance related. Furthermore, increases of test substance related skeletal developmental variations consisting of reduced ossification were noted in the 18 mg/kg/day group. Therefore, these skeletal developmental variations were considered indicative of developmental delay and secondary to the test substance-related effect on fetal growth in the presence of maternal toxicity. Intrauterine growth and fetal morphology at 2 and 5 mg/kg/day and intrauterine survival at 2, 5, and 18 mg/kg/day were unaffected by test substance administration. No external malformations or developmental variations were noted at any dosage level. Other effects: Based on moribundity, clinical observations of rales, gasping, red material on the nose, mouth,		

coronal slice. Soft tissue: Yes, Heads from one-half of the fetuses in each group sectioned for soft tissue evaluation.	and/or forelimbs, mean body weight loss and lower mean body weight gains with corresponding reduced food consumption noted at 18 mg/kg/day, a dosage level of 5 mg/kg/day was considered to be the no observed adverse effect level (NOAEL) for maternal toxicity. Furthermore, increased occurrences of fetal skeletal developmental variations of reduction ossification that were secondary to the growth retardation were observed at 18 mg/kg/day; therefore, a dosage level of 5 mg/kg/day was considered to be the NOAEL for embryo/fetal development when 2-Methyl-1,2 benzisothiazolin 3 one Technical was administered orally by gavage to pregnant Crl:CD(SD) rats.		
Teratogenicity Study – Rabbit Oral (gavage) Species: Rabbit Strain: New Zealand White [Hra:(NZW)SPF] Test guideline: OECD 414 GLP: yes Number of animals per group: 3 MBIT groups of 25 time mated females Duration of exposure: Rabbit: day 7 to 28 post-mating Postexposure period: On gestation day 29, a laparohysterectomy was performed on each surviving female. 1Concentration: Dosage levels were 2, 5, and 20 mg/kg/day administered at a dosage volume of 5 mL/kg. Dosage levels were selected based on the results of the dose range-finding prenatal developmental toxicity study in rabbits. In the dose range-finding study, all females in the 50 mg/kg/day group were found dead or euthanized prior to the scheduled termination following mean body weight losses and corresponding reductions in mean food consumption. Additionally, excreta-related clinical findings (decreased defecation and/or small feces), reduced mean maternal	NOAEL = 5 mg/kg/day for maternal toxicity and NOAEL = 20 mg/kg/day for embryo-fetal development Maternal toxic Effects In the 20 mg/kg/day group, 1 female was euthanized in extremis on gestation day 21 due to body weight loss and minimal food consumption. This female was noted with decreased defecation beginning on gestation day 9 and continuing until the day of euthanasia. Test substance-related decreased defecation was noted for all females in the 20 mg/kg/day group generally during gestation days 9 through 29. The occurrences of decreased defecation in this group generally coincided with decreased food consumption. Maternal toxicity at the high dose was most likely secondary to dose administration of a bolus of MBIT, a strong irritant, by gavage. Teratogenic/embryo-toxic effects There were higher mean litter	1 (reliable) Experimental result Test material (EC name): 2-methyl-1,2-benzisothiazol-3(2H)-one technical (MBIT) CAS-No. 2527-66-4. Purity: 99.68% a.i.	Stump D.G. (2009c)

body weights and food consumption, and lower mean fetal weights were noted at 10 and 25 mg/kg/day. Based on these results, dosage levels of 2, 5, and 20 mg/kg/day were selected for this developmental toxicity study. Body weight: Yes, body weights recorded at appropriate intervals. Individual maternal body weights were recorded on gestation days 0 (by supplier), 4, and 7-29 (daily). Food consumption: Yes, food consumption recorded at appropriate intervals. Individual food consumption was recorded on gestation days 4-29 (daily). Clinical signs: Yes, All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. The rabbits were observed twice daily for general changes in appearance and behaviour. Examination of uterine content: The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. Number of corpora lutea were recorded. Number of total implantations were recorded. Examination of foetuses: The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations. General Fetal weights recorded; external fetal morphological examination followed by fresh dissection. Heads from all fetuses examined by a mid coronal slice. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate, and external orifices,	proportions of 13th full rib(s) and 27 presacral vertebrae noted in the 20 mg/kg/day group. While these increases were considered test substance-related, they are among the more common skeletal developmental variations, occurred in the absence of other indicators of developmental toxicity and are commonly observed in adult rabbits and therefore, were not considered adverse. There were no test substance-related morphological malformations or developmental.		

and each finding was recorded. Nonviable fetuses (if the degree of autolysis was minimal or absent) were examined, the crown rump length measured, weighed, sexed, and tagged individually. Crown rump measurements, degrees of autolysis, and gross examinations, if possible, were recorded for late resorptions, and the tissues were discarded. Number of fetuses, number of litters. Skeletal Yes, All fetal carcasses prepared and stained for skeletal examination. Soft tissue: Yes, Heads from all fetuses examined by a mid coronal slice. Heart and major blood vessels were examined.			

4.11.2.1 Non-human information

4.11.2.2 Human information

No relevant information is available.

4.11.3 Other relevant information

No other relevant information is available.

4.11.4 Summary and discussion of reproductive toxicity

Effect on fertility

Multigeneration Reproduction Toxicity Study – Rat Oral (drinking water) - this study was conducted in compliance with OECD Guideline 416, with analytical confirmation of dose levels. There were no guideline deviations. P males and females are also known as F_0 males and females. Three groups of male and female Crl:CD(SD) rats (30/sex/group) were exposed to the test substance, MBIT, on a continuous basis in the drinking water for at least 70 consecutive days prior to mating. The test substance was offered to the offspring selected to become the F_1 parental generation following weaning (beginning on PND 28). For both the F_1 and F_2 generations, 8 pups per litter (4 per sex, when possible) were selected on PND 4 to reduce the variability among the litters. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F_1 rats.

Nonselected F_1 pups were necropsied on PND 21, and F_2 pups were necropsied on PND 21. Each surviving F_0 and F_1 parental animal received a complete detailed gross necropsy following the completion of weaning of the F_1 and F_2 pups, respectively; selected organs were weighed.

All F_1 offspring selected to constitute the F_1 generation remained with the F_0 dams until lactation day 28 in order to assist pup survival in the presence of excessive maternal and pup toxicity in the 800/400 ppm group. Thirty male and 30 female F_1 pups from each group (control, 50, 200 and 800/400 ppm) were randomly selected prior to weaning to comprise the F_1 generation. These pups (a minimum of 1 male and 1 female per litter, when available) were exposed to the test substance beginning on PND 28.

F_0 and F_1 parental survival was unaffected by the test substance at all exposure levels. Test substance-related clinical observations were limited to the F_0 females in the 200 and 800/400 ppm groups. These findings included increased incidences of red and yellow material on various body surfaces in the 800/400 ppm group primarily during the period of exposure to 800 ppm. Additionally, several females in this same group were noted with an unkempt appearance during lactation days 15-20. These findings were attributed to test substance exposure and were considered adverse. As a result of the reductions in mean body weight gain, mean body weights were up to 15.0% (F_0 males), 23.0% (F_1 males), 6.2% (F_0 females) and 22.8% (F_1 females) lower than the control group throughout the generation for males and during the pre-mating period for females. Lower mean body weights continued to be observed for the F_0 and F_1 females throughout gestation (up to 6.3% and 14.3%, respectively) and lactation (up to 24.6% and 15.9%, respectively).

Corresponding reductions in food consumption were noted for F_0 animals in the 800/400 ppm group and F_1 animals in the 400 ppm group throughout the generation for males and generally throughout the pre-mating, gestation and lactation periods for the females.

The decreases in mean water consumption were believed to be due to the palatability of the test water and were only associated with decreased body weights at the 800/400 ppm exposure level for F_0 parental animals and the 200 and 400 ppm exposure levels for F_1 parental animals.

No test substance-related effects were observed on the mean numbers of F_1 or F_2 pups born, the pup sex ratio or pup survival during the pre-weaning period at any exposure level. There were no test substance-related macroscopic findings for F_0 or F_1 parental animals or in F_1 and F_2 pups at any exposure level. With the exception of lower mean absolute and relative spleen and thymus weights noted for F_1 males and females in the 800/400 ppm group on PND 21, differences in mean F_0 and F_1 parental and F_1 and F_2 pup organ weights from the control group were attributed to reduced mean final body weights and/or were not of a magnitude that would be considered toxicologically significant.

There were no treatment-related microscopic changes observed in any of reproductive organs of the F_0 and F_1 male and female rats designated for evaluation at any exposure level. However, a low incidence of focal papillary edema was observed in F_0 males and females in the 800/400 ppm and in F_1 males in the 400 ppm group. This was associated with an increased incidence and severity of focal cortical and medullary tubular nephropathy in the F_0 males in this group.

Excessive reductions in mean F_0 parental and F_1 pup body weights at 800 ppm indicated that this exposure level would have precluded the objectives of the study. After 16 weeks of exposure at 800 ppm, the exposure level was lowered to 400 ppm. Based on the lack of effects on F_0 and F_1 reproductive performance (mating, fertility, copulation and conception indices, estrous cyclicity and spermatogenic endpoints) , an exposure level of 400 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for parental reproductive toxicity of MBIT when offered in drinking water to Crl:CD(SD) rats. The 800/400 ppm exposure level was equivalent to 40-113 mg/kg/day for the F_0 generation at the 800 ppm exposure level, 20-76 mg/kg/day for the F_0 generation at the 400 ppm exposure level and 23-83 mg/kg/day at the 400 ppm exposure level for the F_1 generation. Parental toxicity was evidenced by reduced mean body weights, body weight gains and food consumption in the F_0 males and females in the 800/400 group and in the and in the F_1 males and females in the 400 ppm group, in addition to red and yellow material findings on various body surfaces and corresponding findings of unkempt appearance for F_0 females in the 800/400 ppm group. Moreover, microscopic findings of focal papillary edema were observed in F_0 males and females in the 800/400 ppm group and in F_1 males in the 400 ppm group, which were judged to be related to exposure. Based on these results, an exposure level of 200 ppm was considered to be the NOAEL for parental systemic toxicity; this exposure level was equivalent to 10-45 mg/kg/day for the F_0 and F_1 generations (and for the pre-mating periods was equivalent to 14 to 22 mg/kg/day). Based on reduced mean pup body weights and body weight gains in the F_1 pups in the 800/400 ppm group and in the F_2 pups in the 400 ppm group, in addition to clinical findings of uneven hair growth and unkempt appearance in F_1 pups in the 800/400 ppm group, the NOAEL for neonatal toxicity was considered to be 200 ppm.

Developmental toxicity

Teratogenicity Study – Rat Oral (gavage) - in the 18 mg/kg/day group, 3/25 females were euthanized *in extremis* on gestation day 9 or 11 due to body weight losses, reduced food consumption, and poor clinical condition. All other females survived to the scheduled necropsy on gestation day 20. Test substance-related clinical observations noted for females euthanized *in extremis* or at the scheduled termination in the 18 mg/kg/day group included rales, gasping, limbs and/or body cool to touch, red material on the nose, mouth, and/or forelimbs, and/or yellow material on the anogenital or urogenital areas at the daily examinations and/or approximately 1 hour following dose administration generally beginning with the initiation of treatment and continuing until euthanasia. These findings were most likely associated with the locally irritating nature of the test substance and not a result of direct systemic toxicity. There were no test substance-related clinical findings noted in the 2 and 5 mg/kg/day groups.

Test substance-related mean body weight losses and lower mean body weight gains with corresponding reductions in mean food consumption were noted in the 18 mg/kg/day group throughout the entire treatment period. As a result, mean body weights in this group were up to 11.6% lower than the control group during gestation days 7-20 and mean net body weight and net body weight gain were lower than the control group value. In addition, mean gravid uterine weight in the 18 mg/kg/day group was lower than the control group and corresponded to the lower mean fetal

weights observed in this group. Mean maternal body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, and food consumption in the 2 and 5 mg/kg/day groups were unaffected by test substance administration.

Four females in the 18 mg/kg/day group (2 of which were euthanized *in extremis*) were noted with gas filled stomach and/or intestines (duodenum, jejunum, ileum, cecum, and/or colon). These findings were likely associated with the reduced food consumption (≤ 14 g/animal) noted for these females approximately 5-6 days prior to the scheduled necropsy/euthanasia and were considered to be a secondary effect test substance administration. With the exception of 1 female each in the control, 2, and 18 mg/kg/day groups, all females were determined to be gravid. No other remarkable macroscopic findings were noted for females in the 2 and 5 mg/kg/day groups.

Mean fetal weights in the 18 mg/kg/day group were 7.9% (male) and 10.5% (female) lower than the concurrent control group values and were considered to be test substance-related Furthermore, increases of test substance-related skeletal developmental variations consisting of reduced ossification were noted in the 18 mg/kg/day group. Therefore, these skeletal developmental variations were considered indicative of developmental delay and secondary to the test substance-related effect on fetal growth in the presence of maternal toxicity. Intrauterine growth and fetal morphology at 2 and 5 mg/kg/day and intrauterine survival at 2, 5, and 18 mg/kg/day were unaffected by test substance administration.

Based on moribundity, clinical observations of rales, gasping, red material on the nose, mouth, and/or forelimbs, mean body weight loss and lower mean body weight gains with corresponding reduced food consumption noted at 18 mg/kg/day, a dosage level of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity.

Furthermore, increased occurrences of fetal skeletal developmental variations of reduction ossification that were secondary to the growth retardation were observed at 18 mg/kg/day; therefore, a dosage level of 5 mg/kg/day was considered to be the NOAEL for embryo/fetal development when 2-methyl-1,2-benzisothiazol-3(2H)-one Technical was administered orally by gavage to pregnant Crl:CD(SD) rats.

Dose administration of MBIT, a strong irritant, by gavage predominately influenced the spectrum of toxicity at the high dose.

Teratogenicity Study – Rabbit Oral (gavage) - one female from the 20 mg/kg/day group that delivered on gestation day 29 was considered within the normal range of delivery days for this species. One female each in the control and 5 mg/kg/day groups was found dead on gestation days 16 and 23, respectively; the cause of death for these females was determined to be intubation error. One female in the control group aborted on gestation day 26. All other females in the control, 2, 5, and 20 mg/kg/day groups survived to the scheduled necropsy; there were no test substance-related internal findings at any dosage level.

There were no test substance-related clinical observations noted in the 2 and 5 mg/kg/day groups.

A test substance-related mean body weight loss, with corresponding reduced food consumption, was noted in the 20 mg/kg/day group during the first week of treatment. Mean body weight gain and food consumption in this group were similar to the control group during the remainder of the treatment period. However, as a result of

the test substance-related effects during the first week of treatment, a lower mean body weight gain and reduced food consumption were noted for the 20 mg/kg/day group when the overall treatment period was evaluated, and mean body weights were up to 8.7% lower than the control group during gestation. There were no test substance-related effects on gravid uterine weights in the 2, 5, and 20 mg/kg/day groups or on body weights, body weight gains, net body weights, net body weight gains, or food consumption in the 2 and 5 mg/kg/day groups.

Intrauterine growth and survival were unaffected by test substance administration at all dosage levels. There were higher mean litter proportions of 13th full rib(s) and 27 presacral vertebrae noted in the 20 mg/kg/day. While these increases were considered test substance-related, they are among the more common skeletal developmental variations, occurred in the absence of other indicators of developmental toxicity and are commonly observed in adults rabbits and therefore, were not considered adverse. There were no test substance-related morphological malformations or developmental variations noted in the 2 and 5 mg/kg/day groups.

Mean body weight losses, reduced food consumption with corresponding decreased defecation, and lower mean body weights resulting in euthanasia of 1 animal were noted in the 20 mg/kg/day group. These findings were considered secondary to the irritant nature of the test substance. Based on these results, a dosage level of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. There was no evidence of developmental toxicity at any dosage level; therefore, a dosage level of 20 mg/kg/day was considered to be the NOAEL for prenatal developmental toxicity when 2-methyl-1,2-benzisothiazol-3(2H)-one Technical was administered orally by gavage to pregnant New Zealand White rabbits.

In summary, MBIT does not show any adverse effects on sexual function and fertility in adult males and females or developmental toxicity in the offspring. MBIT has not to be classified as reproductive toxicant.

4.11.5 Comparison with criteria

No evidence of a reproductive toxicity could be established.

4.11.6 Conclusions on classification and labelling

Classification and labelling is not required.

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal DS proposed no classification for fertility or developmental effects. This conclusion is based on a two generation reproduction toxicity study in rat (OECD TG 416) and developmental toxicity studies in rats and rabbits.

Fertility

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

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

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

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

Developmental toxicity

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

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

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

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

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

Comments received during public consultation

There were no specific comments on reproductive toxicity.

Assessment and comparison with the classification criteria

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

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

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

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

In rabbits, the highest dose, 20 mg/kg bw/d, resulted in reduced maternal weight gain (according to the data given in IUCLID file, body weight changes during GD 7-29 (mean ± SD) for 0, 2, 5, 20 mg/kg bw/d were 145 ± 215.3, 199 ± 175.9, 211 ± 165.3, 37 ± 148.4, respectively). Reduced weight gain was mainly due to body weight losses during the first week of gestation. Food consumption was statistically significantly reduced and also reduced defecation was observed at the high dose. No effects on foetal survival, intrauterine growth or incidence of malformations or visceral variations were observed at any dose level. Incidence of 13th full rib was increased in the high dose group (incidences in 0, 2, 5 and 20 mg/kg bw/d were 42, 79, 72 and 105, respectively) as well as incidences of 27th pre-sacral vertebrae (incidences in 0, 2, 5 and 20 mg/kg bw/d were 16, 25, 23, 34, respectively). On the other hand, incidence of rudimentary 13th rib was lower in high dose group when compared to the controls (incidences in 0, 2, 5 and 20 mg/kg bw/d 22, 30, 27 and 11). RAC concurs with the DS that these variations are very common in New Zealand White rabbits and in the absence of

any other developmental toxic findings they are considered as minimal adversity. In addition, it is noted that these findings were observed only at the highest dose which resulted in reduced food consumption, reduced defecation (which in rabbits may result in malnutrition) and reduced body weight gain. Therefore, RAC does not consider these effects as specific indications of teratogenic potential of MBIT. Since rat developmental toxicity study did not show specific developmental toxicity, either, RAC concurs with DS and proposes no classification for developmental effects.

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

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No studies on neurotoxicity submitted.

There is no evidence in multiple-dose toxicity studies conducted with MBIT (or other compounds within this isothiazolone chemical class) that suggests this compound is neurotoxic. In addition to the lack of evidence pertaining to neurotoxicity, the structure MBIT does not contain any structural features that have, in the past, been associated with neurotoxic compounds. Similarly, there is no evidence in the isothiazolone chemistry, as a whole, that suggests that this class has the potential to produce neurotoxicity in animals or humans.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

No data available.

4.12.2 Summary and discussion

No neurotoxicity and immunotoxicity studies were performed with MBIT.

4.12.3 Comparison with criteria

Not relevant for MBIT.

4.12.4 Conclusions on classification and labelling

No classification required.

RAC evaluation of aspiration toxicity
Summary of the Dossier Submitter's proposal No data were included in the Background Document. Comments received during public consultation No comments were received. Assessment and comparison with the classification criteria No classification is proposed due to lack of data.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

Hydrolytic degradation

The tier one test examined the stability of the test compound at pH 4, 7, and 9 for 5 days at 50°C. If the compound is stable, no further testing is required.

MBIT was found to be hydrolytically stable at an elevated temperature. In pH 4, 7, and 9 buffers no significant hydrolysis of MBIT was observed after 5 days of incubation at 50°C. As a result, the compound is considered hydrolytically stable and no additional tiered testing is required. MBIT comprised 95% or greater after the 5 day incubation (MacLean, S. and Roberts, G.C. (2007)).

Photochemical degradation in water

The phototransformation in water including identity of transformation products were performed according OECD Guideline for testing of Chemicals: "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis".

An initial screen employing the UV/VIS spectrum showed that MBIT could substantially photodegrade so additional testing was performed. A preliminary kinetic test was performed by adding pH 7 phosphate buffer to a test vessel, dosing at 1 µg/ml MBIT, and irradiating the sample using a xenon lamp. The solution was analyzed on Hours 0, 2, and 24. The results showed that additional testing was warranted.

A definitive photolysis study was undertaken by preparing photolysis vessels with pH 7 phosphate buffer. The vessels were dosed at 1 µg/ml and maintained in a bath at 20 ± 3°C. Irradiation was accomplished using a xenon lamp. Duplicate samples were removed at 0, 8, 24, 48, 72, 120, and 168 hours. Samples were radioassayed and aliquots chromatographed (HPLC) to quantitate parent and photodegradates. Photodegradates were identified by LC-MS.

Chromatographic analysis showed that there was a quick loss of MBIT with increasing irradiation time. MBIT decreased from 100% at time 0 to 4% after 168 hours of irradiation (Table 40).

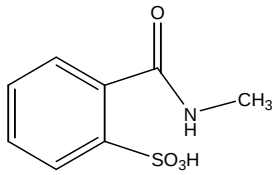
Table 46: Quantitation of MBIT and Photodegradation Products

Photodegradate	**Percent of Applied as MBIT or Transformation Products at Sample Intervals (hrs)**						
	0	**8**	**24**	**48**	**72**	**120**	**168**
1			0.8	1.9	3.8	5.0	8.6
2			2.6	5.6	6.5	4.6	3.3
3			1.1	2.0	2.7	1.4	2.7
4				3.7	6.4	13.4	16.0
5			8.3	10.5	8.2	4.8	3.4
6		4.1	5.2	10.0	16.3	20.2	24.1
7		2.7	6.9	8.3	6.6	3.6	1.7
8					1.6	1.3	1.4
9					1.1	1.5	1.8
MBIT	100	81.9	58.8	33.7	19.0	8.2	4.0
10			1.8	3.5	4.3	5.2	5.8
11			2.0	3.7	4.2	5.4	5.4

There were 11 detected photoproducts. Nine of these photoproducts were more polar than MBIT. Photoproduct 5 reached 10.5% after 24 hours of irradiation but declined quickly to 3.4% after 168 hours. Thus photoproduct 5 is transient.

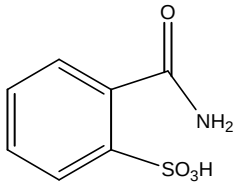
Two photodegradates were detected at greater than 10% after 168 hours. Using LC-MS Transformation Products 4 and 6 were identified as described below:

- 2-(methylcarbamoyl)-benzene sulfonic acid



2-(methylcarbamoyl)-benzenesulfonic acid

- 2-(carbamoyl)-benzene sulfonic acid



2-Carbamoyl-benzenesulfonic acid

Under a Xenon lamp which simulates sunlight, MBIT quickly photodegrades. Photodegradation of MBIT involves cleavage of the isothaizolone ring and subsequent oxidation (MacLean, S.A., Trollope, H.T., and Roberts, G.C. (2008)).

Air phototransformation

The phototransformation rate constant of MBIT is calculated using Structure Activity Relationship (SAR) method (Guo, I. (2009)).

Due to relative low vapor pressure and high water solubility, the concentration of MBIT in the troposphere is expected to be low. This ensures that the photodegradation of the radicals with MBIT follows a pseudo first-order kinetics required by SAR calculation method.

Due to the presence of nitrogen and sulfur bonds, MBIT has a large phototransformation rate constant. The parent compound quickly photodegrades during the daylight with half-life of 13.4 hours.

All potential photodegradation products are expected to be very reactive to photodegradation with half-lives ranging from 1.48 – 472.8 hours.

Daylight photolysis is the dominant phototransformation procedure for MBIT and its potential metabolites.

MBIT photodegrades quickly with half-life of 14.3 hours and the half-lives of its metabolites range from 1.48 – 472.8 hours.

Due to very low production and usage volume, the effect from MBIT and its potential photodegradation products towards global warming is minimal. Therefore, MBIT and its photodegradation metabolites impose no effect to global warming.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Ready biodegradability test of MBIT was determined using a modified OECD 301B, CO_2 Evolution (Modified Sturm Test). Vessels containing mineral salts solution (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NH_4Cl, $MgSO_4$, $CaCl_2$, and $FeCl_3$) plus activated sludge inoculum were prepared. The following systems were prepared: triplicate test vessels containing nominal concentrations of ^{14}C-MBIT at either 1 µg/L or 0.389 mg/L, duplicate reference control vessels containing ^{14}C glucose, and a duplicate toxicity control vessel containing ^{14}C-glucose and MBIT at both dosing concentrations. All vessels were aerated and purged with CO_2-free air. Evolved $^{14}CO_2$ from the test vessels was trapped in NaOH. All vessels were incubated in the dark at 20 ± 2°C. On Days 1, 2, 3, 6, 10, 15, 21, and 28 the traps from test vessels treated with MBIT, reference control, and toxicity control, were refreshed and aliquots of the solutions were removed for quantitation by either liquid scintillation spectroscopy or titration. Solutions from MBIT treated test vessels specifically prepared for parent quantitation and metabolite identification were also prepared. Metabolites were identified by LC-MS.

Less than 1% of the applied activity in the vessels treated with only 14C-MBIT was detected as $^{14}CO_2$. For a compound to be considered ready biodegradable, it must achieve 60% biodegradation to CO_2 and thus MBIT cannot be thus considered. MBIT at nominal concentrations of 1 µg/L and 0.389 mg/L had no observable effect on the microbial activity since ^{14}C-glucose rapidly biodegraded in the presence of ^{12}C-MBIT with CO_2 evolution exceeding 65% by Day 10.

On Day 28, no MBIT was detected in the supernatant. The half-life of MBIT in this system is estimated at 2.1 days or less. Although MBIT can not be classified as ready biodegradable, it does quick undergo primary biodegradation in this system (Daniel, M. (2007)).

As part of a traditional OECD 301B ready test, additional vessels containing 14C-MBIT were prepared. After 28 days these test vessels were then analyzed by LC-MS in order to evaluate the metabolic pathway. Two major metabolites were present:

N-methyl 2-(methylthio)benzamide: ~75% of the activity

Hydroxy-2-methylsulfinyl-benzamide

or

N-methyl-2-(methylsulfinyl)benzamide ~25% of the activity

This study fulfills the requirements and demonstrates that MBIT undergoes quick primary biodegradation having a half-life of 2.1 days or less. Additionally two major

metabolites were identified: N-methyl 2-(methylthio)benzamide and either Hydroxy-2-methylsulfinyl-benzamide or N-methyl-2-(methylsulfinyl)benzamide.

5.1.2.3 Simulation tests

Simulation tests: biodegradation in water

Aquatic biodegradation simulation test in freshwater was conducted according to the OECD Guideline for the Testing of Chemicals 309: Aerobic Mineralization in Surface Water -Simulation Biodegradation Test (Commander, R.F. Oteyza, T. (2009)).

Bottles containing either 500 ml or 1400 ml of fresh surface water were dosed with 14C-MBIT at 10 ppb, 97 ppb, or 1000 ppb. The samples were placed in a dark incubator at 20 ± 2°C. A vacuum was applied to help maintain aerobic conditions and remove volatiles which were trapped in NaOH and ORBO® tubes. Abiotic controls were prepared by adding $HgCl_2$ to the water and then autoclaving prior to the addition of 10 ppb or 100 ppb 14C-MBIT. Reference controls, to validate that there was satisfactory microbial activity, were prepared similar to the MBIT test vessels except they were dosed with 10 ppb sodium benzoate.

From duplicate test vessels containing 1400 ml of MBIT at 10 ppb and 97 ppb, aliquots were removed at 0, 1, 3, and 6 hours and at 1, 2, 7, and 14 days. Water was acidified and applied to an SPE cartridge. The cartridge was eluted with 2% formic acid in water followed by 5% NH_4OH in methanol. The eluants were radioassayed, concentrated and chromatographed by TLC. These 4 bottles were left intact until Day 28 in order to monitor mineralization. The NaOH traps were radioassayed periodically and the ORBO traps were extracted at the termination of the study. A series of vessels containing 500 ml of water and dosed with 10, 100, or 1000 ppb 14C-MBIT were prepared for mass balance analysis on Day 14. Also prepared where the abiotic and reference controls which were also analyzed for mass balance on Day 14. Solutions from the 1000 ppb dosing level were used for LC-MS analysis.

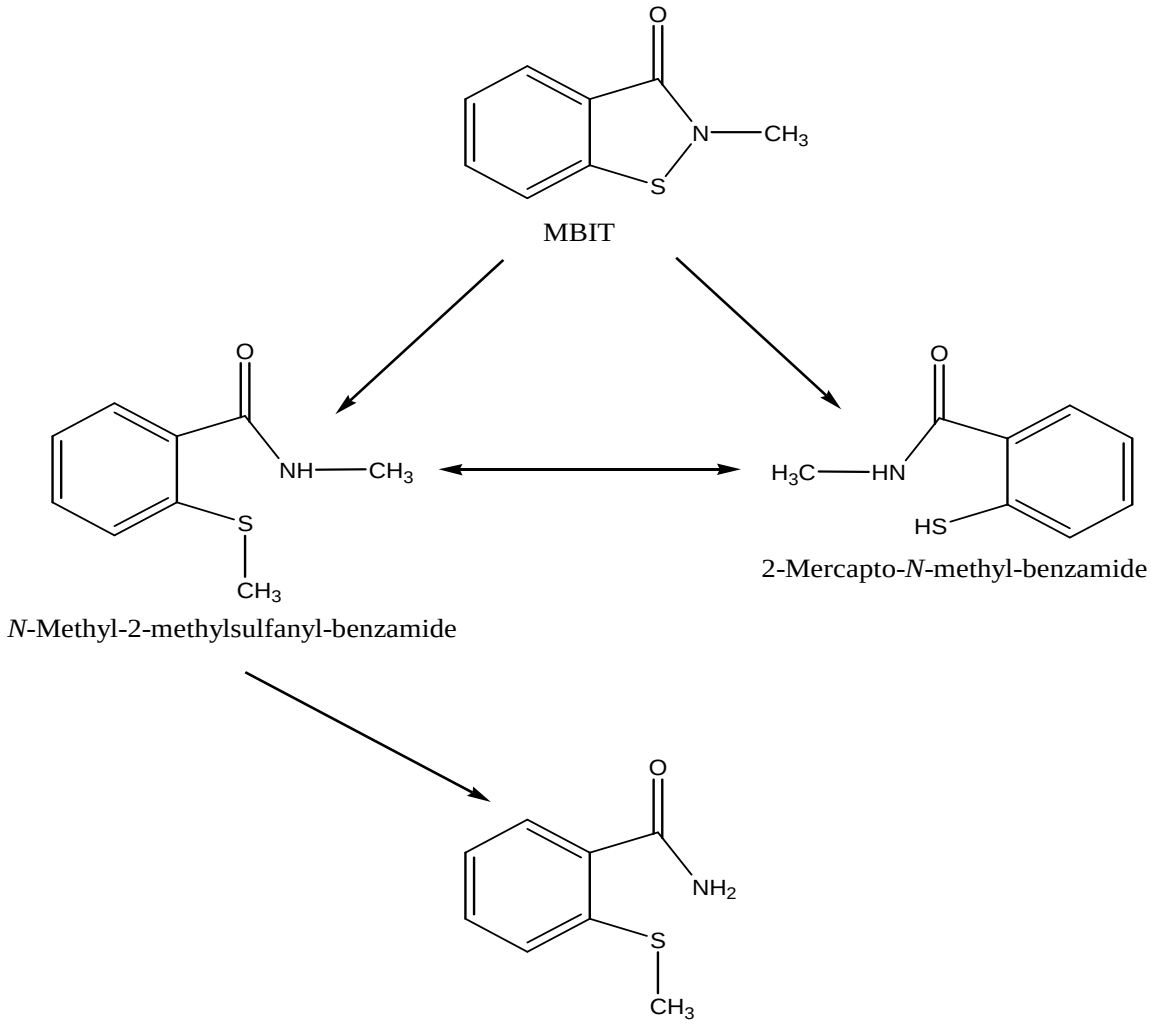
MBIT biodegrades very quickly in the fresh surface water studied. The half-live were 0.34 hrs at 10 ppb and 0.61 hours at 97 ppb. The worst-case DT_{50} value of 0.61 hours at 20°C, equivalent to 0.05 days at environmental temperature is considered to be representative for MBIT in surface water.

Mass balance for non-sterile vessels dosed with ^{14}CMBIT averaged 91.5% while for sterile vessels, 95.7%. Recovery for the benzoic acid reference vessel was 89.4%. The total average recovery of applied activity was 91.9%. For vessels treated with ^{14}C-MBIT less than 1% of the applied activity was present as $^{14}CO_2$ on Day 14. Benzoic acid treated vessels averaged about 52% evolved $^{14}CO_2$ on Day 14 demonstrating that the water was microbially active. There were essentially no volatile organics produced based on the analysis of the Orbo™ traps. Parent degraded rapidly such that in the test vessels, after 24 hrs, less than 8% of applied activity was parent. One metabolite was identified as N-methyl-2-(methylthio)-benzamide. A second metabolite was identified as either/or both 2-Methylsulfanyl-benzamide or 2-Mercapto-N-methyl benzamide. Both have the same empirical formula and molecular

mass (a third possibility was 2-Methylsulfanyl-benzimidic acid but this is a very unlikely structure).

Similar to the results in other media MBIT degrades in fresh water. The half-life was less than 1 hour. Metabolism involved cleavage of the isothiazolone ring.

Figure 1: Metabolic Pathway of MBIT in Surface Water



Simulation tests: sewage treatment plant

Aerobic sewage treatment simulation test was performed according to OECD Guidaline 303: Simulation Test-Aerobic Sewage Treatment: Activated Sludge (Schaefer, E.C., Cartee, R.T., and Carpenter, K. (2009).

The test unit was a porous pot bioreactor which consists of a glass vessel housing a polyethylene membrane that retains the sludge solids but allows the liquid to flow through. Three reactors were prepared; a control dosed with water and two test reactors dosed with ^{14}C-MBIT. 1.13L of activated sludge was added to the reactors and domestic sewage was pumped into the system at 2.4 ml/min. A 2.35 mg/L

solution of ^{14}C-MBIT was added to the porous pot system at a flow rate of 0.3 ml/min for a resulting concentration in the porous pot of 0.25 mg/L. About 113 ml of activated sludge was removed per day. The hydraulic retention time in the aeration vessel was 7 hours and the sludge retention time, 10 days. The effluent was collected in a refrigerated container.

The unit was allowed to equilibrate (stabilization period) for 8 days prior to dosing with ^{14}C-MBIT during which the DOC/COD became greater than 8%. A 12 day acclimation period followed the stabilization period and during this time the systems were dosed with MBIT (the control with a similar volume of water). The effluent, mixed liquor and dosing solution were radioassayed. After 12 days the system had reached equilibrium and a 21 day steady test period was commenced. During the steady test period, the effluent, mixed liquor, mixed liquor supernatant, acetonitrile extract of the mixed liquor solids, and dosing solution were radioassayed. The system temperature was maintained at 20 ± 2C.

Dissolved organic carbon, pH, temperature, and oxygen content were monitored throughout the study.

During the steady test period volatile traps consisting of KOH were connected to the effluent to collect evolved $^{14}CO_2$. Aliquots of the KOH were taken periodically for radioassay.

The effluent and an acetonitrile extract of the sludge solids were chromatographed using HPLC.

In a sewage treatment plant simulation system dosed with ^{14}C-MBIT about 74% of the applied activity was in the effluent and 20% in the mixed liquor. Evolved CO_2 totaled less than 0.1% of the total applied radioactivity.

The half-life of MBIT in the simulated STP systems was 0.32 hours.

MBIT was present at less than 7% of the applied activity.

5.1.3 Summary and discussion of degradation

Table 47: Summary of relevant information on degradation.

Method	Results	Reference
Hydrolysis OECD Guideline 111 Hydrolysis as a function of pH GLP: yes	The stability of the test compound at pH 4, 7, and 9 for 5 days at 50°C was examined. No significant hydrolysis of MBIT was observed. k_H - not determined since MBIT was stable at pH 4, 7, and 9. DT_{50} - not determined since MBIT was stable at pH 4, 7, and 9.	MacLean, S. and Roberts, G.C. (2007)
Phototransformation in water OECD Guideline for testing of Chemicals: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis GLP: yes	An initial screen employing the UV/VIS spectrum showed that MBIT could substantially photodegrade so additional testing was performed. Results: - Measured rate constant, kc = 0.5382 days-1 resulting in a half-life of 1.3 days - Quantum yield, øc = 1.65 x 10-5	MacLean, S.A., Trollope, H.T., and Roberts, G.C. (2008)

	- Theoretical maximum rate constant, kc(max) Summer = 91 days-1 Winter = 869 days-1 - Environmental rate constants, kc(env) Summer = 0.014 day-1 resulting in a half-life of 50 days Winter = 0.0015 day-1 resulting in a half-life of 462 days Two photodegradates were detected at greater than 10% after 168 hours - 2-(methylcarbamoyl)-benzene sulfonic acid - 2-(carbamoyl)-benzene sulfonic acid In a definitive photolysis study chromatographic analysis showed that there was a quick loss of MBIT with increasing irradiation time. MBIT decreased from 100% at time 0 to 4% after 168 hours of irradiation.	
Screening test Ready biodegradability test of MBIT was determined using a modified OECD 301B, CO_2 Evolution (Modified Sturm Test). GLP: yes	Less than 1% of the applied activity in the vessels treated with only 14C-MBIT was detected as $14CO_2$. For a compound to be considered ready biodegradable, it must achieve 60% biodegradation to CO_2 and thus MBIT cannot be thus considered. MBIT undergoes quick primary biodegradation having a half-life of 2.1 days or less	Daniel, M. (2007)
Simulation tests Aquatic biodegradation simulation test in freshwater was conducted according to the OECD Guideline for the Testing of Chemicals 309.	MBIT biodegrades very quickly in the fresh surface water studied. The half-live were 0.34 hrs at 10 ppb and 0.61 hours at 97 ppb. The worst-case DT_{50} value of 0.61 hours at 20°C, equivalent to 0.05 days at environmental temperature is considered to be representative for MBIT in surface water.	Commander, R.F. Oteyza, T. (2009)
Simulation tests Aerobic sewage treatment simulation test was performed according to OECD Guidaline 303.	The half-life of MBIT in the simulated STP systems was 0.32 hours.	Schaefer, E.C., Cartee, R.T., and Carpenter, K. (2009)

In a ready biodegradation studies, MBIT was found **not to be ready biodegradable**. Nevertheless, biological half-lives in the environment are very short:
- the half-life of MBIT in ready biodegradability test is estimated to be less than 2.2 days,
- MBIT biodegrades very quickly in the fresh surface water studied. The half-live were 0.05 days at 12°C,
- the half-life of MBIT in the simulated Sewage Treatment Plant (STP) system was 0.32 hour.

Metabolism of degradation involved cleavage of the isothiazolone ring.
The short half-life implies that the concentration of parent compound in the environment will be low.

Simulation tests show rapid primary biodegradation of MBIT in the environment. According to the Guidance on the Application of CLP criteria (Version 4.1 – June 2015) data on primary degradation can only be used to show rapid degradation of substance where it is demonstrated that the degradation products shall not be classified as hazardous to the environment, i.e. that they do not fulfil the classification criteria. Main metabolites identified during degradation of MBIT are:
- N-Methyl-2-(Methylthio)Benzamide,
- 2-(methylcarbamoyl)- benzene sulfonic acid,
-2carbamoyl- benzene sulfonic acid.

The ready biodegradability test and aquatic acute tests were performed with two of these metabolites.
In the Table presented below the results of these studies are summarized:

Table 48. The results of environmental studies performed with metabolites of MBIT.

Method	Results	Remarks	Reference
N-Methyl-2-(Methylthio)Benzamide			
Determination of ready biodegradability according to the OECD guidelines 301F: Manometric respirometry	Biodegradation of N-Methyl-2-(methylthio)benzamide in the Test Suspensions reached **only 5.4% over the 28-day test period** (as determined by BOD). This result indicates that very little biodegradation did occur under the conditions of the test and the 60% criterion for classification as "readily biodegradable," according to the OECD 301F: Manometric Respirometry Test (OECD, 1992) was not met.	N-methyl-2-(methylthio)benzamide Purity: 99.7 GLP	Chai, Y., and Hales, C.A.. (2014)
An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Guideline: OECD 203	The LC_{50} value for N-Methyl-2-(methylthio)benzamide exposed to the freshwater fish, Rainbow trout **was >101 mg a.i./L**, the highest concentration tested.	N-methyl-2-(methylthio)benzamide Purity: 99.7 GLP	Currie, R.J., Hutchinson, K.L., Holzheuer, B.S. (2014a)
An Acute Toxicity Study with the Freshwater *Cladoceran, Daphnia magna.* Guideline: OECD 202	Based on the results, N-methyl-2-(methylthio)benzamide was not acutely toxic to daphnids, with an **EC_{50} value of >101 mg/L** (104 mg/L measured), the highest concentration tested.	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 99.7 GLP	Currie, R.J., Hutchinson, K.L., and Holzheuer, W.B., (2014b)
Growth inhibition test with the freshwater green alga, *Pseudokirchneriella subcapitata.* Guideline: OECD 201	96 hour **E_rC_{50} >101 mg a.i./L**	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 99.7 GLP	Currie, R.J., Hutchinson, K.L., and Holzheuer, W.B.. (2014c)
2-(methylcarbamoyl)- benzene sulfonic acid			
Determination of ready biodegradability according to the OECD guidelines 301F: Manometric respirometry test	Biodegradation of 2-(methylcarbamoyl)-benzene sulfonic acid in the Test Suspensions indicated a total of **8.3% biodegradation over the 28-day test period** (as determined by BOD). This value indicates that very little biodegradation did occur under the conditions of the test and the 60% criterion for classification as "readily biodegradable," according to the OECD 301F: Manometric Respirometry Test (OECD, 1992) was not met.	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 98.5 GLP	Chai, Y., and Hales, C.A.. (2014)
An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss.* Guideline: OECD 203	2-(methylcarbamoyl)-benzene sulfonic acid LC_{50} in the freshwater fish, Rainbow trout **was >101 mg a.i./L,** the highest concentration tested.	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 98.5 GLP	Currie, R.J., Louch, D.W., Holzheuer, B.S. (2014a)
An Acute Toxicity Study with the Freshwater Cladoceran, Daphnia magna. Guideline: OECD 202	Results for 2-(methylcarbamoyl)-benzene sulfonic acid indicate no acute toxicity to *Daphnia magna* at 101 mg/L (103 mg/L measured), the highest concentration tested.	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 98.5 GLP	Currie, R.J., Louch, D.W., and Holzheuer, W.B., (2014b)
Growth inhibition test with the freshwater *green alga,* Pseudokirchneriella subcapitata. Guideline: OECD 201	96 hour **E_rC_{50}: >101 mg a.i./L**. Confidence intervals could not be determined	Sodium 2-(Methylcarbamoyl)Benzenesulfonatate Purity: 98.5 GLP	Currie, R.J., Hutchinson, K.L., and Holzheuer, W.B.. (2014c)

Taking into account the results of environmental tests performed on metabolites of MBIT it can be concluded that:

- N-Methyl-2-(Methylthio)Benzamide should not be classified, according to CLP Regulation, as hazardous to the environment (the substance is not readily biodegradable but the results of aquatic acute tests performed on fish, daphnia and algae are higher than 100 mg/l – see classification criteria, Table 4.1.0 b) (iii) Annex I of CLP).

- 2-(methylcarbamoyl)-benzene–sulfonic acid should not be classified, according to CLP, as hazardous to the environment (the substance is not readily biodegradable but the results of aquatic acute tests performed on fish, daphnia and algae are higher than 100 mg/L – see classification criteria, Table 4.1.0 b) (iii) Annex I of CLP).

Taking into account the fact that in general metabolites are much less toxic than MBIT, and that chemical structure and QSAR properties of 2-(methylcarbamoyl)-benzene –sulfonic acid and -2-carbamoyl-benzene –sulfonic acid are very similar (see Table 48 – QSAR Estimated Values for MBIT metabolites), it can be assumed that conclusions presented for 2-(methylcarbamoyl)-benzene –sulfonic acid are also relevant for 2-carbamoyl-benzene –sulfonic acid.

According to the EPIWIN QSAR modelling results, all MBIT degradates have been shown to be low in toxicity to aquatic organisms and not likely to bioaccumulate. In case of Metabolite#1 and Metabolite#2 estimated results are in line with the results from submitted ecotoxicological studies. From ecotoxicological point of view it can be therefore assumed that MBIT metabolites are out of concern.

Table 49. QSAR Estimated Values for MBIT Metabolites.

Chemical Name	2-(methylcarbamoyl)-benzene sulfonic acid (Metabolite #2)	2-carbamoyl-benzene sulfonic acid (Metabolite#3)	N-methyl-2-(methylthio)-benzamide (Metabolite #1)
SMILES Notation	O=C(c1ccccc1S(=O)(O)=O)NC	NC(=O)(c1ccccc1S(=O)(O)=O)	O=C(c1ccccc1S(C)=O)NC
Molecular Weight	215.23	201.20	181.25
Vapor Pressure (Pa at 25ºC)	3.45×10^{-8}	6.60×10^{-8}	1.94×10^{-3}
Water Solubility (at 25ºC, $mg \cdot L^{-1}$)	1×10^{6}	1×10^{6}	2.28×10^{3}
Log K_{OW} (at 25ºC, -)	-1.954	-3.1551	1.8048
K_{OC} (at 25ºC, -)	10	10	46.74
Ready Biodegradability Primary Biodegradation Ultimate Biodegradation	No (No) Days Weeks	No Days Weeks	No (No) Days Weeks-Months
Fish 96-h LC_{50} ($mg \cdot L^{-1}$)	>>1000 (>101)	3.09×10^{6}	33.3 (>101)
Daphnid 48-h EC_{50} ($mg \cdot L^{-1}$)	>>1000 (>101)	2.94×10^{5}	17.5 (>101)

Green algae 96-h EC_{50} ($mg \cdot L^{-1}$)	59.8 (>101)	20.708	0.451 (>101)

Conclusions:
- simulation tests show rapid primary biodegradation of MBIT in the environment,
- the degradation products (N-Methyl-2-(Methylthio)Benzamide, 2-(methylcarbamoyl)-benzene –sulfonic acid and 2-carbamoyl-benzene –sulfonic acid are not classified as hazardous to the environment.

Taking into account all available data it can be concluded that MBIT is rapidly degradable for the purposes of aquatic hazard classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Adsorption/desorption from soil and sediment study was performed according to the test OECD 106 guideline - Adsorption-Desorption Using a Batch Equilibrium Method (Marbo, M. (2008)). The four soils and 1 sediment were gamma irradiated prior to dosing because preliminary work had demonstrated the instability of MBIT in the soil:$CaCl_2$ test system.

A series of studies were initially performed to establish the test conditions such as adsorption to the test vessel, appropriate soil: $CaCl_2$ solution ratio, and equilibration time. The potential of MBIT to adsorb to the test vessel was examined by dosing 0.01M $CaCl_2$ in the test vessel and radioassaying the supernatant.

The effect of the ratio of soil to 0.01M $CaCl_2$ solution was examined. A series of soil: $CaCl_2$ solution ratios were examined for each soil. Soil and $CaCl_2$ were equilibrated by shaking overnight and the next morning ^{14}C-MBIT was added. The mixture was shaken for 24 hours, centrifuged, and the supernatant radioassayed.

A study to determine the time necessary to reach adsorption equilibration was performed by adding soil and 0.01M$CaCl_2$ in the appropriate determined ratio and mixing overnight. ^{14}C-MBIT was added at 100 µg/ml and duplicate tubes removed, centrifuged, and the solution radioassayed at 2, 4, 6, 24, and 48 hours. The supernatants were also chromatographed as were the methanol soil extracts. The desorption equilibrium was determined by removing the ^{14}C solution after an adsorption period and replacing it with ^{14}C-free 0.01M $CaCl_2$. The test vessels were centrifuged at 2, 4, 6, 24, and 48 hours and aliquots removed for radioassay.

A study to determine the time necessary to reach adsorption equilibration was performed by adding soil and 0.01M$CaCl_2$ in the appropriate determined ratio and mixing overnight. ^{14}C-MBIT was added at 100 µg/ml and duplicate tubes removed, centrifuged, and the solution radioassayed at 2, 4, 6, 24, and 48 hours. The supernatants were also chromatographed as were the methanol soil extracts. The desorption equilibrium was determined by removing the ^{14}C solution after an adsorption period and replacing it with ^{14}C-free 0.01M $CaCl_2$. The test vessels were centrifuged at 2, 4, 6, 24, and 48 hours and aliquots removed for radioassay.

The definitive adsorption isotherm study was performed with a soil:0.01M $CaCl_2$ solution ratio and equilibration times determined in the above studies. The appropriate amount of soil and volume of $CaCl_2$ solution were added to Teflon® centrifuged tubes, mixed overnight, and then the ^{14}C-MBIT added at 5 ppm, 50 ppm, 125 ppm, 250 ppm, or 500 ppm. Tubes were shaken for 6-24 hour, centrifuged, and the supernatant radioassayed. After the applicable adsorption equilibrium, a desorption process was initiated by removing the ^{14}C-$CaCl_2$ solution and adding an equal volume of ^{14}C-free-$CaCl_2$ solution. After 24 hrs of shaking, the test vessels were removed, centrifuged, the supernatant removed, and radioassayed.

MBIT remained relatively stable throughout the testing interval. Without gamma sterilization of the soil, MBIT will degrade significantly in the test system.

The study provided is satisfactory to describe the mobility of MBIT in soil. According to the US EPA classification scheme, MBIT is considered high to medium mobility. It is highly likely that in the environment, MBIT will be rapidly degraded before it can leach and be an environmental concern.

Soil adsorption test according to OECD Guideline 312 was also performed - Leaching in Soil Columns (Noble, H.L. and Trollope, H.T. (2009)). 50 g soil (dry weight) was added to sealed glass vessels and after equilibration at 20 ± 2°C, several were dosed at 1 ppm ^{14}C-MBIT. After 27.33 hrs two flasks were removed and extracted sequentially with 0.005M $CaCl_2$, 0.005M CaCl2:35 g/L NaCl, and methanol. The extracts and remaining soil residues were radioassayed. An aliquot of the any extract containing greater than 10% of the applied activity was analyzed by TLC and LC-MS.

Leaching columns were prepared by placing sieved sandy loam soil into duplicate glass segmented (5 cm id x 5 cm height) columns. The overall column length was 35 cm with 30 cm packed with soil. The columns were prewetted with 0.005M $CaCl_2$. To the top of two columns, ^{14}C-MBIT dosed soil aged for about 27 hours was added to the top 5 cm segment. To two additional columns, control soil aged for about 27 hours was added and then ^{14}C-MBIT added to this soil. Leaching was accomplished by adding 313 ml of 0.005M $CaCl_2$ over a 48 hour period and the leachate collected. At the conclusion, the soil column was separated into segments (6 column segments, 1 aged soil segment). The leachate and soil segments were radioassayed. Soil segments containing more than 10% of the applied activity were extracted with 0.1N NaOH and any extract and leachate fractions containing more than 10% of the applied activity were analyzed by TLC.

^{14}C-MBIT dosed soil was aged for 27.33 hours. This was the estimated half-life derived in preliminary soil metabolism study. Results from the definitive soil metabolism study yielded a half-life of less than 2 hours.

About 67%-69% of the applied activity was detected in the soil segments and about 30% in the leachate. Of the activity detected in the soil, about 50% was in the top 5 cm which contained ^{14}C-MBIT aged soil or control aged soil subsequently dosed with ^{14}C-MBIT. In general there was a decrease in activity with increasing column depth. Results from columns where the aged residue was placed on the top of the soil column and those where the top segment contained aged control soil which was dosed

directly with ^{14}C-MBIT are essentially the same (no difference between aged and non-aged soils).

Samples of ^{14}C-MBIT aged soil similar to that placed on top of two columns was extracted and subsequently analyzed by TLC. Eleven degadates were detected and two of them exceeded 10% of the applied activity. Aliquots of the two degradates greater than 10% of the applied activity were also analyzed by LC-MS but due to the low concentration positive confirmation was not possible. However, one peak had a mass that correlated with 2-(methylcarbamoyl)benzene sulfonic acid.

Aliquots of the leachate were initially analyzed by TLC. There were 4 degradates detected. Two of the degradates exceeded 10% of the applied activity and they were chromatographically similar to the two major metabolites in the extracted soil. Analysis of the two major leachate degradates by LC-MS failed to yield structural information due to the low concentration. There was no MBIT detected in the leachate indicating that MBIT will not leach appreciably and should not be persistent in the environment.

This study confirms the quick degradation of MBIT in soil and the resulting fast half-life (<2 hrs). Probably due to degradation, MBIT shows limited mobility within soil column and should not be persistent in ground water. No MBIT was detected in the leachate. Two major degradates were detected in the aged soil and leachate and they are chromatographically similar. Due to the low concentration of the degradates LC-MS was not able to supply any structural information. However one peak had a mass that correlated with 2-(methylcarbamoyl)benzene sulfonic acid.

5.2.2 Volatilisation

Due to relative low vapour pressure and high water solubility, the concentration of MBIT in the troposphere is expected to be low.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

No data. The log P (log octanol:water partition coefficient) for this compound is < 1.6. This value indicates that the potential for MBIT to bioaccumulate will be minimal.

5.3.1.1 Bioaccumulation estimation

MBIT has a log Kow < 1.6.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The risk of bioaccumulation of MBIT is neglible.

5.4 Aquatic toxicity

Table 50: Summary of relevant information on aquatic toxicity.

Method	Results	Remarks	Reference
OECD Guideline 203	LC50: 0.24 mg a.i./L	96 hours flow-through conditions Rainbow trout (Oncorhynchus mykiss)	Sayers L.E., (2007a)
OECD Guideline 203	LC50: 1.5 mg a.i./L	96 hours flow-through conditions Sheepshead minnow (Cyprinodon variegatus)	Soucy K. (2009a)
OECD Guideline 202	48 h EC50: 0.92 mg a.i./L	48 hours flow-through conditions Daphnia magna	Sayers L.E., (2007b)
US EPA OPPTS draft guideline 850.1035 and US EPA OPPTS 850.1000	96 h LC50: 0.48 mg a.i./L	96 hours flow-through Mysid (Americamysis bahia)	Soucy K. (2009b)
OECD Guideline 201	48 h ErC50 of 0.24 mg a./L NOErC of 0.012 mg a.i./L	96 hours Fresh water-Pseudokirchneriella subcapitata	Hoberg J.R., (2007)
OECD Guideline 201	96 hour EC50: 0.75 mg a.i./L (95% C.I.: 0.69 – 0.82 mg a.i./L) 96 hour NOEC: 0.48 mg a.i./L	96 hours Marine water Skeletonema costatum	Softcheck K.A. (2009)
OECD Guideline 210	32-day NOEC: 0.16 mg a.i./L	32 day duration flow-through Fathead minnow	Hamitou M. (2009a)
OECD Guideline 211	21-day NOEC: 0.42 mg a.i./L (survival)	21 days flow-through Daphnia magna	Hamitou M. (2009b)
US EPA OPPTS 850.1735 and ASTM Guideline 1706-05	NOEC: 50 mg MBIT/kg (Based on growth; and 10 days survival) LOEC:> 50 mg MBIT/kg based on growth; 99 mg MBIT/kg (Based on 10 days survival) LC50: 100 mg MBIT/kg (Based on 10 days survival), >50 mg MBIT/kg based on growth	Acute flow-through toxicity study Fresh water Midge larvae, (Chironomus dilutus)	Bradley M.J. (2009a)
OECD Guideline 218	NOEC: 66 mg MBIT/kg (Based on midge emergence and development rate) LOEC: >66 mg MBIT/kg (Based on midge emergence and development rate)	28 days Fresh water Midge larvae, (Chironomus riparius)	Bradley M.J. (2009b)

	EC50: >66 mg MBIT/kg		

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Acute toxicity tests were performed on:
- rainbow trout (*Oncorhynchus mykiss*),
- sheepshead minnow (*Cyprinodon variegatus*).

An acute toxicity test with rainbow trout was conducted in compliance with OECD Guideline 203 with analytical confirmation of dosing concentrations at test initiation and at 96 hours of exposure. There were no guideline deviations. Temperature, pH and dissolved oxygen were measured daily during the test and were within acceptable limits. The 96 h LC_{50} for *O. mykiss* of 0.24 mg a.i./l is based on mean measured concentrations (Mortality data and Effect data are presented in tables below).

Table 51: Mortality data

Test-Substance Concentration (measured)[1] [mg a.i./L]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control (water)	0/10	0/10	0/10	0/10	--	--	--	--
Solvent control (acetone)	0/10	0/10	0/10	0/10	0	0	0	0
0.019	0/10	0/10	0/10	0/10	0	0	0	0
0.050	0/10	0/10	0/10	0/10	0	0	0	0
0.11	0/10	0/10	0/10	0/10	0	0	0	0
0.22	4/10	4/10	4/10	4/10	40	40	40	40
0.46	10/10	10/10	10/10	10/10	100	100	100	100
Temperature [°C]	11	12	11-12	11-12				
pH	6.4-6.6	6.4-6.6	6.7-6.8	6.5-6.6				
Oxygen [mg/l]	9.0-9.8	8.4-9.2	9.2-9.9	9.3-9.9				

[1] specify, if TS concentrations were nominal or measured

Table 52: Effect data

	48 h [mg a.i./l][1]	**95 % c.i.**	**96 h [mg a.i./l][1]**	**95 % c.i.**
LC_0	0.11 (m)	No data	0.11 (m)	No data
LC_{50}	0.24 (m)	0.11 to 0.46	0.24 (m)	0.11 to 0.46
LC_{100}	0.46 (m)	No data	0.46 (m)	No data

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

An acute toxicity test with Sheepshead minnow was conducted in compliance with OECD Guideline 203 with analytical confirmation of test solution concentrations. There were no guideline deviations. Test fish were maintained under the following conditions (16 hours light, 8 hours darkness, 20% salinity seawater, fed flake fish food and brine shrimp) for 14 days prior to test initiation in a 20 litre glass aquaria. The MBIT dose levels for the definitive exposure study are based on the results of preliminary flow-through and static exposures with MBIT dose groups.

Following 24 hours of exposure, 100% mortality was observed among fish exposed to the 2.2 and 4.3 mg a.i./L treatment levels. Following 96 hours of exposure, no mortality or adverse effects were observed among fish exposed to the remaining treatment levels tested (0.33, 0.40 or 1.0 mg a.i./L) or the controls. The 96 h LC_{50} for *Cyprinodon variegatus* of 1.5 mg a.i./l is based on mean measured concentrations (Mortality data and Effect data are presented in tables below).

Table 53: Mortality data

Test-Substance Concentration (measured)[1] [mg a.i./L]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control (seawater)	0/10	0/10	0/10	0/10	0	0	0	0
Solvent control (acetone)	0/10	0/10	0/10	0/10	0	0	0	0
0.33	0/10	0/10	0/10	0/10	0	0	0	0
0.40	0/10	0/10	0/10	0/10	0	0	0	0
1.0	0/10	0/10	0/10	0/10	0	0	0	0
2.2	10/10	10/10	10/10	10/10	100	100	100	100
4.3	10/10	10/10	10/10	10/10	100	100	100	100
Temperature [°C]	22-23	23	22	22				
pH	7.5-7.7	7.6-7.7	7.6-7.7	7.6-7.7				
Oxygen [mg/l]	6.3-8.2	6.1-7.9	6.7-8.2	6.8-8.1				

[1] specify, if TS concentrations were nominal or measured

Table 54: Effect data

	48 h [mg/l][1]	**95 % c.l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	1.0 mg a.i./L (m)	Not available	1.0 mg a.i./L (m)	Not available
LC_{50}	1.5 mg a.i./L (m)	1.0 to 2.2 mg a.i./L	1.5 mg a.i./L (m)	1.0 to 2.2 mg a.i./L
LC_{100}	2.2 mg a.i./L (m)	Not available	2.2 mg a.i./L (m)	Not available

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

5.4.1.2 Long-term toxicity to fish

Long-term toxicity (Early life-stage toxicity test) with Fathead minnow was conducted in compliance with OECD Guideline 210 with analytical confirmation of dose levels. There were no guideline deviations.

Dead and live embryos were counted daily until hatching was complete. Day of hatch was considered to be exposure day 4, when no more than 10% unhatched viable embryos remained in the control egg incubation cup. During the post-hatch exposure period larval fish were observed daily and the behaviour and appearance of the larval fish were recorded and dead larvae were removed. At 28 days post-hatch exposure (experimental completion), the percentage of fish survival was determined. The surviving fish were euthanized with tricaine and measured and weighed individually to determine the total length and dry weight.

Total hardness, alkalinity and specific conductivity were monitored at experimental start and on test days 6, 10, 20, 27 and 30 in one replicate of the highest treatment level and the dilution water control during the exposure.

Calculations of percentage survival of organisms at hatch were based on the number of live, dead and deformed larvae per incubation cup after hatching was complete (test day 4 / day 0 post-hatch) compared to the number of embryos per cup on test day 1.

Based on the results of this study, embryo hatching success, number of normal fry at hatch and survival at test termination were the most sensitive indicators of toxicity of 2-Methyl-1,2-benzisothiazol-3(2H)-one to fathead minnow. The NOEC and LOEC for fathead minnow were determined to be 0.16 and 0.39 mg a.i./L, respectively. The EC_{50} values for embryo hatching success, number of normal fry at hatch and survival at test termination were calculated to be 0.33 mg a.i./L (95% confidence interval: 0.31 to 0.35 mg a.i./L) and 0.37 mg a.i./L (95% confidence interval: 0.35 to 0.39 mg a.i./L), respectively.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Acute toxicity tests were performed on:

- *daphnia magna*,

- mysid (*Americamysis bahia*).

An acute toxicity test was performed with daphnia magna in compliance with OECD Guideline 202 with analytical confirmation of dosing concentrations at test initiation and at 48 hours of exposure. There were no guideline deviations. Based on the results of a 48-hour preliminary range-finding study, the nominal concentrations for the definitive study were selected. Temperature, pH and dissolved oxygen were measured daily during the test (0, 24 and 48 hours) and were within acceptable limits. Dissolved oxygen ranged from 8.5 to 9.3mg/L and exceeded 60% saturation. The 48 hours EC_{50} for *daphnia magna* of 0.92 mg a.i./l is based on mean measured concentrations (Immobilisation data and Effect data are presented in tables below).

Table 55: Immobilisation data

Test-Substance Concentration (measured) [1] **[mg a.i./L]**	**Immobile *Daphnia***						
	Number		**Percentage**		**Oxygen [mg/l]**	**pH**	**Temperature [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
Control (water)	0/20	0/20	0	0	9.1-9.2	7.9	21
Solvent control (acetone)	0/20	0/20	0	0	9.0-9.1	7.9	21
0.24	0/20	0/20	0	0	8.6-8.7	7.9	21
0.65	0/20	0/20	0	0	8.8-8.9	7.9	21
1.3	1/20	20/20	5	100	8.8-8.9	7.9	21
2.8	4/20	18/20	20	90	8.8-8.9	7.9	21
6.1	20/20	20/20	100	100	8.6-8.7		21

[1] specify, if TS concentrations were nominal or measured

Table 56: Effect data

	EC_{50}[1]	**95 % c.i.**	**EC_0[1]**	**EC_{100}[1]**
24 h [mg a.i./L]	3.3 (m)	2.7 to 4.0	0.65 (m)	6.1 (m)
48 h [mg a.i./L]	0.92 (m)	0.65 to 1.3	0.65 (m)	1.3 (m)

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

An acute toxicity test was performed with mysid in compliance with US EPA OPPTS draft guideline 850.1035 and US EPA OPPTS 850.1000 with analytical confirmation of dosing concentrations at test initiation and at 96 hours of exposure. There were no guideline deviations. Twenty mysids were exposed to each MBIT treatment level and controls. Toxicity endpoints were based on mean measured concentrations. Following the 72 hours and 96 hours of exposure, 100% mortality was observed among mysids exposed to the 0.88 mg a.i./L treatment level. Following 96 hours of exposure, 5% and 25% mortality was observed among mysids exposed to the 0.21 and 0.43 mg a.i./L treatment levels, respectively. Several surviving mysids exposed to the 0.43 mg a.i./L treatment level were observed to be lethargic at test termination. No mortality or adverse effects were observed among mysids exposed to the remaining treatment levels tested (0.043 and 0.095 mg a.i./L), the seawater control or the solvent control (acetone). The 96 h LC_{50} for *mysids* of 0.48 mg a.i./l is based on mean measured concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Daphnia magna chronic reproduction study was conducted in compliance with OECD Guideline 211 with analytical confirmation of dose levels. There were no guideline deviations.
The number of immobilised adult daphnids and observations of abnormal behaviour were recorded daily. Assessments of offspring released were determined on test days 10, 12, 14, 17, 19 and 21. In addition, the number of immobilised offspring and the time to first brood release were recorded.

At test completion (day 21), total body length of each surviving adult daphnid was measured. The diluter system was monitored daily.

There was no significant reduction in offspring per female among daphnids exposed to MBIT concentrations when compared to the pooled controls. There was no significant reduction in mean total body length in daphnids exposed to MBIT concentrations when compared to the pooled controls. The most sensitive parameter was survival of daphnids.

Based on the most sensitive parameter (survival), the 21-day NOEC and LOEC values for 2-Methyl-1,2-benzisothiazolin-3-one and Daphnia magna were determined to be 0.42 and 0.95 mg a.i./L, respectively. The EC_{50} value for survival was calculated to be 0.70 mg a.i./L (95% confidence interval: 0.60– 0.78 mg a.i./L).

5.4.3 Algae and aquatic plants

Two studies were conducted to determine toxicity of MBIT to freshwater and saltwater algae . Tests were conducted according to OECD 201 and US EPA OPPTS 850.5400 guidelines on two species *Pseudokirchneriella subcapitata* and *Skeletonema costatum* – detail information about these studies are included in Annex 1 of CLH report. In both tests algae were exposed to MBIT for 72 hrs and 96 hrs in the static system. Calculation of endpoints in these studies is problematic. This problem concerns all isothiazolinones (including MBIT), which present a special case and might therefore warrant a deviation from guidelines.
Data on MBIT toxicity to algae were re-assessed by in the same manner as it had been done for other isothiazolinones. First of all validity criteria were re-checked according to criteria given in OECD 201 guideline. Secondly in reliable studies, actual concentration of MBIT was carefully analysed during the tests. At last endpoints of each study have been assessed day-by-day to check which time period in the study is the most sensitive for MBIT. Finally, depending on this relevant period, endpoints for MBIT were proposed - as the initial measured concentrations (if the most sensitive period was 0-24 hours) or by using TWA approach (if the most sensitive period was not 0-24 hours). Presented assessment follows not standard approach. Usually, according to OECD 201 guideline, 72 hours intervals are used for endpoints determination. However, as other isothiazolinones, MBIT may be expected to have unique mode of action in algae. Therefore this special assessment is needed.

Study with *Pseudokirchneriella subcapitata* was considered to be reliable. All criteria in this test were met with exception of criterion 2 for 0-96 hr period. For this reason 0-96 hour period was not considered as relevant for endpoint determinations. In addition high differences among control replicates were identified during the first 24 hr period

(24.3%). Consequently, NOEC values was considered to be not relevant for this time period and thus EC_{10} were determined additionally.
During study concentration of MBIT declined in all test systems and was not maintained at >80% of nominal concentrations during the test. Analysis of samples collected at 96 hours showed that concentration of MBIT decreased to <LOQ in most of test concentrations with the exception nominal concentration of 0.16, 0.40 and 1.0 mg a.i./L, which had measured concentrations of 8, 13 and 68% of nominal concentration at study termination, respectively. For this reason actual concentrations based rate constant (k) were derived and then the TWA concentrations were calculated.
It should be also pointed that concentration of test substance declined in all test systems, but the most pronounced in the test systems with the lower test concentrations which is typical for algal tests with isothiazolinones. It is important to distinguish that the degradation of the isothiazolinones in the test is due to their reactivity with the test organisms, which also accounts for the toxicity. The higher the inhibition of algal growth, the slower the degradation of MBIT is the test medium.
To identify the most sensitive period E_rC_{50} and E_rC_{10} values were calculated on the basis of initial measured concentrations by using Hill model in Regtox.EV7.06 software. NOE_rC values have been derived from Dunett`s test (5%).
The period 48h was identified as the most sensitive for MBIT. E_rC_{10} and NOE_rC values were during this period the lowest. Finally, according to WG IV/2016 agreements NOEC, (0.012 mg a.i/L) with value lower than E_rC_{10}, (0.09 mg a.i/L) was considered to be the most representative endpoint for MBIT. Based on TWA concentrations of MBIT, 48-h E_rC_{50} is 0.24 mg/L.

Study with *Skeletonema costatum* was consider to be not reliable. The second OECD 201 criterion in control cultures was not met. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3) in a control cultures was 46% thus it exceed the trigger value of 35%. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3 and 3-4) in a control cultures was 55%, thus it also exceed the trigger value of 35%. What more important for this study, the second OECD 201 criterion in solvent control cultures was also not met. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3) in a control cultures was 51% thus it exceed the trigger value of 35%. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3 and 3-4) in a control cultures was 67%, thus it also exceed the trigger value of 35%. In addition it should be also pointed that validity criteria were not fulfilled for 0-1 and 1-2 days – the mean CV was above 35% (49% in a control cultures and 68% in a solvent control cultures).

5.4.4 Other aquatic organisms (including sediment)

Toxicity to sediment dwelling organisms

Acute sediment toxicity test was conducted with in compliance with larvae of *Chironomus dilutus* (Bradley M.J. (2009)). Based on nominal sediment concentrations and midge survival, the LOEC and NOEC were determined to be 100 and 50 mg a.i./kg, respectively. Based on nominal sediment concentrations and midge growth, the LOEC and NOEC were determined to be >50 and 50 mg a.i./kg, respectively. Based on nominal concentrations, the 10-day LC_{50} value for midge survival was

determined to be 99 mg a.i./kg with 95% confidence intervals of 89 to 100 mg a.i./kg. Since no concentration tested resulted ≥ 50% reduction in ash-free dry weight, the EC_{50} value for midge growth was empirically estimated to be > 50 mg a.i./kg the highest concentration statistically analyzed.

Chronic sediment toxicity test was conducted with larvae *of Chironomus riparius* (Bradley M.J. (2009)) according to OECD Guideline 218. Midges were exposed to MBIT concentrations for 28 days under static test conditions (based on the results of preliminary testing Midges were exposed to MBIT at nominal concentrations of 6.3, 13, 25, 50 and 100 mg a.i./kg. Based on mean measured concentrations, the three highest treatment levels were defined as 3.6, 14, and 39 mg a.i./L).

Artificial sediment composition: 2.4% organic carbon, 80% sand, 3% silt, 17% sand, 73% solids at 6.7 pH. Measurements of dissolved oxygen concentration, temperature and pH were made on day 0 and day 28 in each exposure vessel. In addition, dissolved oxygen concentration and temperature were measured daily in an alternating vessel for each treatment levels and the controls. Exposure concentrations of MBIT were measured on day 0 (test initiation), day 7 and day 28 (test termination) in the overlying water, pore water and sediment. The results of this study are based on nominal concentrations of applied TS ad are presented based on dry weight. Overlying water used in this study was laboratory well water. Test vessels were examined daily. Observations of midge emergence and abnormal behaviour were recorded. During the period of expected emergence (typically starting at day 10 and lasting until day 28), a daily check of emerged midges was made. The sex and number of adult midges that emerged daily were recorded. Male midges were identified by their plumose antennae. The development rate of male, female, and male and female midge combined, was determined. Mean development time represents the time span between the addition of test organisms (day 0) and the emergence of experimental midges. The stock solution was observed to be clear and colourless with no visible undissolved test substance.

Since no concentration tested during this study reduced emergence or development time by 50% or more, the EC_{50} values were empirically estimated to be greater than the highest concentration tested. All water quality parameters monitored during the study were unaffected by the concentrations of MBIT tested and remained within acceptable limits. Based on the analytical results of sediment, pore water and overlying water during this study, the majority of the 2-Methyl-1,2-benzisothiazol-3(2H)-one that was applied to sediment remained associated with the sediment, but continually degraded through the duration of the exposure.

It should be also pointed that as MBIT degradates in surface waters rapidly (with DT_{50} of 0.05 d at 12ºC) and as in both tests it was indicated that initial measured concentrations of test substance in sediment were below 80% of nominal concentrations, endpoints for MBIT could be derived on the basis of geometric mean measured concentrations.

However, taking into account physico-chemical properties of MBIT (log Kow of 1.4 and Koc 153 L/kg) and its rapid degradation in surface waters this active substance is not likely to adsorb to sediment to a significant extent. Therefore taking into account results from a spiked sediment tests is irrelevant for environmental assessment.

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

CLP – Acute aquatic hazards

The lowest available $L(E)C_{50}$ value relevant for classification of MBIT is the 48 h E_rC_{50} of **0.24 mg a./L** obtained for the *Pseudokirchneriella subcapitata and* 96 h LC_{50} of **0.24 mg a./L** obtained for *Oncorhynchus mykiss.* Based on this lowest $L(E)C_{50}$ values MBIT fulfils the criteria $L(C)E_{50} \leq 1$ mg/L for classification as **Acute Aquatic Category 1, H400** (Very toxic to aquatic life) with **M-factor of 1** due to 48 h E_rC_{50} and 96 h LC_{50} are in the range $0.1 < L(E)C_{50} \leq 1.0$ mg/L.

CLP – Aquatic Chronic hazards

The lowest $NOEC/EC_{10}$ is the 48 hours **NOE_rC of 0.012 mg a.i./L** obtained for freshwater alga species *Pseudokirchneriella subcapitata.* Available NOEC values for fish and Daphnia are higher. The lowest endpoint for MBIT for algae fulfils the criteria 0.01 mg/l > $NOEC/ECx \leq 0.1$ mg/L (for substance readily biodegradable – Table 4.1.0 b) (ii)) for classification as **Aquatic Chronic 2, H411** (Toxic to the aquatic organisms with long lasting effects).

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

In accordance with the provisions of CLP Regulation MBIT should be classified as hazardous to the environment:

- Aquatic Acute 1, H400; M=1

- Aquatic Chronic 2, H411

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier Submitter's proposal The DS proposed that MBIT is rapidly degradable and that it should be classified as: - Aquatic Acute 1; H400 (M-factor of 1) based on a 96-h LC_{50} of 0.24 mg/L for fish and a 48-h E_rC_{50} of 0.24 mg/L for algae. - Aquatic Chronic 2; H411 based on a 48-h NOE_rC of 0.012 mg/L for algae. *Degradation* MBIT is hydrolytically stable over 5 days at 50 °C and pH 4, 7 and 9. This equates to a half-life at 25 °C of >1 year at pH 4, 7 and 9.

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

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

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

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

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

Table: Fate and ecotoxicity data for MBIT degradants

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

subcapitata) OECD TG 201, GLP			
Algal growth inhibition QSAR estimate	96-h EC_{50} = 0.451 mg/L		EPIWIN
Degradant 2: 2-(methylcarbamoyl)-benzene sulfonic acid			
Water solubility (at 25 °C) QSAR estimate	1.0 × 10^6 mg/L		EPIWIN
Log K_{OW} (at 25 °C) QSAR estimate	-1.95		EPIWIN
Ready biodegradation OECD TG 301F, GLP	8.3 % degradation	Not rapidly biodegradable	Chai and Hales, 2014
Ready biodegradation QSAR estimate	Not readily biodegradable		EPIWIN
Acute toxicity to fish (*O. mykiss*) OECD TG 203, GLP	LC_{50} > 101 mg/L		Currie *et al*., 2014a
Acute toxicity to fish QSAR estimate	96-h LC_{50} > 1 000 mg/L		EPIWIN
Acute toxicity to invertebrates (*Daphnia magna*) OECD TG 202, GLP	EC_{50} > 101 mg/L		Currie *et al*., 2014b
Acute toxicity to invertebrates QSAR estimate	48-h EC_{50} > 1 000 mg/L		EPIWIN
Algal growth inhibition (*Pseudokirchneriella subcapitata*) OECD TG 201, GLP	E_rC_{50} > 101 mg/L		Currie *et al*., 2014c
Algal growth inhibition QSAR estimate	96-h EC_{50} = 59.8 mg/L		EPIWIN
Degradant 3: 2-carbomyl-benzene sulfonic acid			
Water solubility (at 25 °C) QSAR estimate	1.0 × 10^6 mg/L		EPIWIN
Log K_{OW} (at 25 °C) QSAR estimate	-3.15		EPIWIN
Ready biodegradation QSAR estimate	Not readily biodegradable		EPIWIN
Acute toxicity to fish QSAR estimate	96-h LC_{50} = 3.09 × 10^6 mg/L		EPIWIN
Acute toxicity to invertebrates QSAR estimate	48-h EC_{50} = 2.94 × 10^5 mg/L		EPIWIN
Algal growth inhibition QSAR estimate	96-h EC_{50} = 20.7 mg/L		EPIWIN

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

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

Bioaccumulation

The octanol-water partition coefficient (log K_{OW}) is < 1.6. No further information is available. This is below the CLP criterion for a bioaccumulative substance (log K_{OW} > 4), so the DS

considered that MBIT does not have potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

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

Table: Summary of relevant information on aquatic toxicity of MBIT

Method	Test organism	Endpoint	Toxicity values (mg/L)	Reference
Short-term toxicity to fish				
OECD TG 203 (flow-through)	*Oncorhynchus mykiss* (Rainbow Trout)	96-h LC_{50}	0.24 (95 % CI: 0.11–0.46)	Sayers, 2007a
OECD TG 203 (flow-through)	*Cyprinodon variegatus* (Sheepshead Minnow)	96-h LC_{50}	1.5 (95 % CI: 1.0–2.2)	Soucy, 2009a
Long-term toxicity to fish				
OECD TG 210 (flow-through)	*Pimephales promelas* (Fathead Minnow)	32-d NOEC	0.16	Hamitou, 2009a
Short-term toxicity to aquatic invertebrates				
OECD TG 202 (flow-through)	*Daphnia magna*	48-h EC_{50}	0.92 (95 % CI: 0.65–1.3)	Sayers, 2007b
US EPA OPPTS 850.1035 (flow-through)	*Americamysis bahia* (mysid shrimp)	96-h LC_{50}	0.48 (95 % CI: not provided)	Soucy, 2009b
Long-term toxicity to aquatic invertebrates				
OECD TG 211 (flow-through)	*Daphnia magna*	21-d $NOEC_{survival}$	0.42	Hamitou, 2009a
Toxicity to algae and aquatic macrophytes[a]				
OECD TG 201 (static)	*Pseudokirchneriella subcapitata*	24-h E_rC_{50} 48-h E_rC_{50} 72-h E_rC_{50}	0.419 (95 % CI: 23.6–27.8) 0.373 (95 % CI: 0.350–0.396) 0.319 (95 % CI: 0.264-0.374) (based on initial measured concentrations)	Hoberg, 2007 and subsequent evaluating Member State (eMS) reanalysis [b]
			<u>Reanalysis by eMS:</u>	
		24-h E_rC_{50} 48-h E_rC_{50} 72-h E_rC_{50}	0.474 (95 % CI: not provided) 0.361 (95 % CI: not provided) 0.315 (95 % CI: not provided) (based on initial measured concentrations)	
		24-h E_rC_{10} 48-h E_rC_{10} 72-h E_rC_{10}	0.334 (95 % CI: 0-22.6) 0.129 (95 % CI: 0.107–0.150) 0.167 (95 % CI: 0.079-0.253) (based on initial measured	

			concentrations) <u>Reanalysis by eMS:</u> 0.321 (95 % CI: not provided) 0.157 (95 % CI: not provided) 0.166 (95 % CI: not provided) (based on initial measured concentrations) 0.16 0.027 0.068 (based on initial measured concentrations) <u>Reanalysis by eMS:</u> 0.16 0.027 0.068 (based on initial measured concentrations) <u>Reanalysis by eMS using mean measured (time-weighted average) concentrations for Biocidal Products Regulation:</u> 0.24 (95 % CI: not provided) 0.09 (95 % CI: not provided) 0.012	
		24-h E_rC_{10} 48-h E_rC_{10} 72-h E_rC_{10} 24-h NOE_rC 48-h NOE_rC 72-h NOE_rC 24-h NOE_rC 48-h NOE_rC 72-h NOE_rC 48-h E_rC_{50} 48-h E_rC_{10} 48-h NOE_rC		
OECD TG 201 (static)	*Skeletonema costatum*		Study results included in CLH Annex but as the study validity criteria were not met, the study is not considered reliable and further details are not included.	Softcheck, 2009 and subsequent evaluating Member State reanalysis

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

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

Comments received during public consultation

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

Hazard labelling

One MSCA stated that labelling with H400 (Very toxic to aquatic life) alone is not appropriate as it does not communicate the long-term hazard. The DS agreed that labelling with H410

(Very toxic to aquatic life with long lasting effects) would cover both the short- and long-term hazards avoiding duplication (in accordance with Article 27 of the CLP Regulation).

Degradation

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

Aquatic toxicity

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

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

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

ii) 96-hour endpoints from the Hoberg (2007) algal study are not reliable, but test guideline validity criteria were met at 48 hours;

iii) chronic fish and *Daphnia* endpoints were based on mean measured concentrations; and

iv) the acute *Chironomus dilutus* study was conducted in compliance with a standard test guideline.

Assessment and comparison with the classification criteria

Degradation

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

Bioaccumulation

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

Acute Aquatic toxicity

Short-term aquatic toxicity data are available for three trophic levels. The lowest endpoints for fish and invertebrates are:

96-h LC_{50} of 0.24 mg/L for *Oncorhynchus mykiss*
96-h LC_{50} of 0.48 mg/L for *Americamysis bahia*

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

As these endpoints are below 1 mg/L, the substance meets the criteria for classification with Aquatic Acute 1. As $0.1 < L(E)C_{50} \leq 1$ mg/L, the M-factor is 1. Therefore RAC supports the DS's proposal.

Chronic Aquatic toxicity

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

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

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

In summary, on the basis of the available data, RAC considers that MBIT should be classified as Aquatic Chronic 2. This is consistent with the conclusion of the DS, but follows a different line of reasoning given the difference in opinion about rapid degradation and interpretation of the algal studies.

Supplemental information - In depth analyses by RAC

The CLH report refers to EPIWIN, but does not indicate which version was used. Furthermore only prediction results were provided, without further documentation (i.e. assessment of the model and whether the substances are within the applicability domain). At the request of the rapporteur, the ECHA secretariat produced its own QSAR analysis for several degradants of MBIT, to provide reassurance about the data reported by the DS. The results are briefly summarised below. Several degradants were detected in the photo-transformation test in water at concentrations above 1 % of the applied amount of MBIT over 168 hours. In addition, two to four additional degradants were detected in the ready biodegradability test and in the simulation test in water but some were not explicitly identified.

Predictions were made with ECOSAR v1.11 using the following QSAR models: Amides, Amides – acid, Phenols, Phenol amines, Thiols and mercaptans.

ECHA noted a mismatch between the chemical name and the SMILES code provided in the CLH report for one of the metabolites, although using the presumably correct SMILES (matching the chemical name given) gave similar results to those provided in the CLH report.

Table: Metabolites and LC50/EC50

Metabolite number	96-h Fish LC_{50} (mg/L)	48-h Daphnia LC_{50} (mg/L)	96-h Algae EC_{50} (mg/L)
1	74	89	2
2	432 000	2 940 000	5 791
3	2 920 000	34 600 000	30 192

4	100	3	53
5	2 865	9 355	54
6	497	1 043	11
7	7	0.8	0.5

In general, the predictions for fish are based on models with a low number of experimental data, but a reasonably high R^2. The models for short-term toxicity to daphnids and algae for these types of substance were built on very few data points and the R^2 for the algae model is rather low (0.2), meaning that the algal predictions cannot be regarded as reliable. However, the models for these types of substance all indicate that the toxicity is somewhat higher than it would be for neutral organics with the same log K_{OW}.

There are no very close structural analogues in the training sets for comparison with the target structures to check the reliability of the predictions. For the fish model, the closest analogue in the training set may be benzamide (CAS no. 55-21-0). The experimental results for this substance are similar to the predictions for fish and daphnid toxicity (the predictions being somewhat more conservative).

ECHA noted that metabolite #7 (2-mercapto-*N*-methyl benzamide) is predicted to be the most toxic, with short-term fish toxicity predicted to be 7 mg/L. Short-term toxicity end points for daphnids and algae are predicted to be even lower (though are uncertain).

As a general observation, RAC considers that the amounts of transformation products being produced over a particular time period are relevant for decisions about lack of rapid degradation. However, neither the CLP Regulation nor the accompanying guidance currently provide any indications about how this should be taken into account. RAC recommends that ECHA considers a guidance update to address this gap.

6 OTHER INFORMATION

This proposal for harmonized classification and labelling is based on the data provided for the registration of MBIT according to Directive 98/8/EC (repealed by Regulation 528/2012). The summaries included in this proposal are partly copied from CAR. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the CAR.

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8 ANNEXES

Annex 1:
Algal studies on MBIT

Data on MBIT toxicity to algae were re-assessed in the same manner as it had been done for other isothiazolinones (DCOIT, MIT, CMIT/MIT and BIT).

Re-assessment presented in this paper were provided by eCA (PL) on the basis of cell density data from study reports provided by Applicant.
First of all validity criteria were re-checked according to criteria given in OECD 201 guideline. Secondly, actual concentration of MBIT was carefully analysed during the tests. At last endpoints of each study have been assessed day-by-day to check which time period in the study is the most sensitive for MBIT. Finally, depending on this relevant period, endpoints for MBIT were proposed - as the initial measured concentrations (if the most sensitive period was 0-24 hours) or as the mean measured concentrations (if the most sensitive period was not 0-24 hours). Assessment presented in this paper follows not standard approach. Usually, according to OECD 201 guideline, 72 hours intervals are used for endpoints determination. However, as other isothiazolinones, MBIT may be expected to have unique mode of action in algae. Therefore this special assessment in needed.
Two tests on algae had been submitted for MBIT. One on a fresh water species *Pseudokirchneriella subcapitata* and one on a marine species *Skeletonema costatum.*

Study on *Pseudokirchneriella subcapitata* – Hoberg, 2007

Validity criteria
According to OECD 201 guideline (adopted in 2006, with Annex 5 corrected in 2011) the following criteria should be in test on algae met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period;
- The mean coefficient of variation for section-by-section growth rates (day 0-1, 1-2, 2-3 for 72-hour tests) in the control cultures must not exceed 35%. This criterion applies to the mean value of coefficient of variation calculated for replicate control cultures;
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures must not exceed 7% in tests on *Pseudokirchneriella subcapitata.*

Table 1. Cell density data in control cultures in study with *Pseudokirchneriella subcapitata*

Cells density (cells/mL)					
Control	**0h**	**24h**	**48h**	**72h**	**96h**
A	10000	35000	360000	1252500	2795000
B	10000	32500	397500	1656700	3095000
C	10000	55000	390000	1320000	2705000
D	10000	80000	417500	1623300	2850000
E	10000	65000	360000	1626700	2940000
F	10000	87500	332500	1423300	2755000
Mean	10000	59167	376250	1483750	2856667

According to results presented in Table 1 the first criterion of OECD 201 guideline was met. The biomass in the control cultures increased exponentially by a factor of 148 within 72-hour period and by a factor of 287 within 96 - hour period.

According to results presented to Table 2 the second OECD 201 criterion was met. Mean coefficient of variation for section- by-section specific growth rates (0-1, 1-2, 2-

3) in a control cultures was 27% thus it did not exceed the trigger value of 35%. However, mean coefficient of variation for section- by-section specific growth rates (0-1, 1-2, 2-3 and 3-4) in a control cultures exceeded the trigger value of 35% - it was 45%.

According to results presented in Table 3 the third OECD 201 criterion was met in controls. During the whole 72hr and 96 hr period the coefficient of variation of average specific growth rates in replicate control cultures did not exceed 7% - it was 2.4 and 0.9% respectively.

In addition it could be indicated that during the 48 hr period the coefficient of variation of average specific growth rates in replicate control cultures was also low (2.3%) (Table 3). High differences among control replicates were however identified during the first 24 hr period (24.3%). Consequently, NOEC values seem to be not relevant for this test and thus EC_{10} were determined additionally.

pH in the control in study by Hoberg (2007) was 7.0 at 0 h, 7.7 at 72h and 9.1 h. Therefore OECD 201 criterion that the pH in the control medium should not increase by more than 1.5 units during the test was met until 72 h.

Assuming all above it can be concluded that study with *Pseudokirchneriella subcapitata* can be considered as reliable (RI =2). All criteria in this test were met with exception of criterion 2 for 0-96 hr period. For this reason 0-96 hour period should not be considered as relevant for further determinations.

Table 2. Average growth rates and coefficient of variations of section-by-section growth rate in study with *Pseudokirchneriella subcapitata* calculated by eCA on the basis of cells density data given in study report

Average growth rate day by day					**0-1;1-2**			**0-1;1-2;2-3**			**0-1;1-2;2-3;3-4**		
Control	**0-24**	**24-48**	**48-72**	**72-96**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**
A	1.25	2.33	1.25	**0.80**	1.79	0.76	0.43	1.61	0.62	0.39	1.41	0.65	0.46
B	1.18	2.50	1.43	**0.62**	1.84	0.94	0.51	1.70	0.70	0.41	1.43	0.79	0.55
C	1.70	1.96	1.22	**0.72**	1.83	0.18	0.10	1.63	0.38	0.23	1.40	0.55	0.39
D	2.08	1.65	1.36	**0.56**	1.87	0.30	0.16	1.70	0.36	0.21	1.41	0.64	0.45
E	1.87	1.71	1.51	**0.59**	1.79	0.11	0.06	1.70	0.18	0.11	1.42	0.57	0.40
F	2.17	1.34	1.45	**0.66**	1.75	0.59	0.34	1.65	0.45	0.27	1.40	0.62	0.44
					mean		**0.27**	**mean**		**0.27**	**mean**		**0.45**

Table 3. Average growth rates and coefficient of variations of growth rate during test period in study with *Pseudokirchneriella subcapitata* calculated by eCA on the basis of cells density data given in study report

Average growth rate during test period				
Control	**0-24 h**	**0-48 h**	**0-72 h**	**0-96 h**
A	1.25	1.79	1.61	1.41
B	1.18	1.84	1.70	1.43
C	1.70	1.83	1.63	1.40
D	2.08	1.87	1.70	1.41
E	1.87	1.79	1.70	1.42
F	2.17	1.75	1.65	1.40
mean	1.71	1.81	1.66	1.41
SD	0.42	0.04	0.04	0.01
CV	**0.243**	**0.023**	**0.024**	**0.009**

Actual concentrations of MBIT in study with *Pseudokirchneriella subcapitata*

Concentration of MBIT in study with *Pseudokirchneriella subcapitata* was measured at the beginning (0h) and at the end of the test (96h) (Table 4).

Table 4. Actual concentrations of MBIT in study with *Pseudokirchneriella subcapitata*

Concentration of MBIT		
Nominal (mg a.i./L)	**Measured (mg a.i./L)**	
	0 h	**96 h**
Control	< LOQ*	< LOQ**
0.0040	0.0043 (108)	< LOQ**
0.010	0.011 (110)	< LOQ**
0.026	0.027 (104)	< LOQ**
0.064	0.068 (106)	< LOQ***
0.16	0.16 (100)	0.012 (8)
0.40	0.42 (105)	0.053 (13)
1.00	1.1 (110)	0.68 (68)

values in brackets represent percentage of nominal concentration
LOQ* = 0.00098 mg a.i./L, LOQ** = 0.0012 mg a.i./L, LOQ***=0.0038 mg a.i./L

Initial measured concentrations of test substance represented 104 -110% of nominal concentrations.

During study this concentration declined in all test systems and was not maintained at >80% of nominal concentrations during the test. Analysis of samples collected at 96 hours showed that concentration of MBIT decreased to <LOQ in most of test concentrations with the exception nominal concentration of 0.16, 0.40 and 1.0 mg a.i./L, which had measured concentrations of 8, 13 and 68% of nominal concentration at study termination, respectively.

For this reason actual concentrations based rate constant (k) were derived and then the mean measured concentrations were calculated (please refer to Table 5).

It should be also pointed that concentration of test substance declined in all test systems, but most pronounced in the test systems with the lower test concentrations which is typical for algal tests with isothiazolinones. It is important to distinguish that the disappearance of the isothiazolinones in the test is due to their reactivity with the test organisms, which also accounts for the toxicity. The higher the inhibition of algal growth, the slower the disappearance of MBIT is the test medium. This makes it

difficult to correctly account for disappearance of MBIT in a TWA approach. It can be anticipated that at least in the two lowest test concentration the actual concentration already dropped below the LOQ within the first 24 hours of the test.

Table 5. Concentrations of MBIT based on rate constant (k) in study with *Pseudokirchneriella subcapitata*

Initial measured concentration (mg a.i./L)	Concentration measured at 96 h (mg a.i./L)	k (1/h)	Concentration calculated at each time (mg a.i./L)				Mean measured concentrations (TWA approach) (mg a.i./L)			
			24h	48h	72h	96h	24h	48h	72h	96h
0.0043	0.0006*	0.021	0.0026	0.0016	0.0010	0.0006	0.003	0.003	0.002	0.0019
0.011	0.0006*	0.030	0.0053	0.0026	0.0012	0.0006	0.008	0.006	0.004	0.0036
0.027	0.0006*	0.040	0.0104	0.0040	0.0016	0.0006	0.017	0.012	0.009	0.0069
0.068	0.0019*	0.037	0.0278	0.0114	0.0046	0.0019	0.045	0.032	0.024	0.0185
0.160	0.012	0.027	0.084	0.044	0.023	0.012	0.118	0.09	0.071	0.0571
0.420	0.053	0.022	0.250	0.149	0.089	0.053	0.328	0.26	0.213	0.1773
1.100	0.68	0.005	0.98	0.86	0.77	0.68	1.036	0.98	0.923	0.8732

* concentration expressed as LOQ/2

The most sensitive period in study with *Pseudokirchneriella subcapitata*

To identify the most sensitive period E_rC_{50} and E_rC_{10} values were calculated on the basis of initial measured concentrations by using Hill model in Regtox.EV7.06 software. NOE_rC values have been derived from Dunett`s test (5%).

Table 6. Cell density data in study with *Pseudokirchneriella subcapitata*

Cells density (cells/mL)					
Control	**0h**	**24h**	**48h**	**72h**	**96h**
A	10000	35000	360000	1252500	2795000
B	10000	32500	397500	1656700	3095000
C	10000	55000	390000	1320000	2705000
D	10000	80000	417500	1623300	2850000
E	10000	65000	360000	1626700	2940000
F	10000	87500	332500	1423300	2755000
0.0043	0h	24h	48h	72h	96h
A	10000	40000	365000	1600000	2935000
B	10000	42500	380000	1287500	2535000
C	10000	70000	350000	1550000	2615000
0.011	0h	24h	48h	72h	96h
A	10000	77500	422500	1390000	2525000
B	10000	82500	365000	1790000	2530000
C	10000	60000	412500	1640000	2365000
0.027	0h	24h	48h	72h	96h
A	10000	87500	352500	1257500	2310000
B	10000	105000	452500	1833300	2375000
C	10000	90000	360000	1393300	2350000
0.068	0h	24h	48h	72h	96h
A	10000	105000	380000	1963300	28250000
B	10000	135000	257500	1403300	2350000
C	10000	65000	275000	1440000	2750000
0.16	0h	24h	48h	72h	96h
A	10000	37500	302500	1127500	2130000
B	10000	55000	252500	805000	2150000
C	10000	50000	250000	947500	2180000
0.42	0h	24h	48h	72h	96h
A	10000	22500	47500	42500	172500
B	10000	25000	50000	50000	132500
C	10000	25000	42500	32500	150000
1.00	0h	24h	48h	72h	96h
A	10000	10000	12500	7500	17500
B	10000	2500	17500	22500	12500
C	10000	15000	15000	7500	15000

Table 7. Growth rates during different time periods in study with *Pseudokirchneriella subcapitata* calculated by eCA (PL) on the basis of cells density data presented in Table 6

0-24 h								
Initial measured conc. (mg a.i./L)	**0**	**0.0043**	**0.011**	**0.027**	**0.068**	**0.16**	**0.42**	**1.00**
Replicates	1.25	1.39	2.05	2.17	2.35	1.32	0.81	0.00
	1.18	1.45	2.11	2.35	2.60	1.70	0.92	-1.39
	1.70	1.95	1.79	2.20	1.87	1.61	0.92	0.41
	2.08							
	1.87							
	2.17							
0-48 h								
Initial measured conc. (mg a.i./L)	**0**	**0.0043**	**0.011**	**0.027**	**0.068**	**0.16**	**0.42**	**1.0**
Replicates	1.79	1.80	1.87	1.78	1.82	1.70	0.78	0.11
	1.84	1.82	1.80	1.91	1.62	1.61	0.80	0.28
	1.83	1.78	1.86	1.79	1.66	1.61	0.72	0.20
	1.87							
	1.79							
	1.75							
0-72 h								
Initial measured conc. (mg a.i./L)	**0**	**0.0043**	**0.011**	**0.027**	**0.068**	**0.16**	**0.42**	**1.0**
Replicates	1.61	1.69	1.64	1.61	1.76	1.58	0.48	-0.10
	1.70	1.62	1.73	1.74	1.65	1.46	0.54	0.27
	1.63	1.68	1.70	1.65	1.66	0.00	0.00	0.00
	1.70							
	1.70							
	1.65							
0-96 h								
Initial measured conc. (mg a.i./L)	**0**	**0.0043**	**0.011**	**0.027**	**0.068**	**0.16**	**0.42**	**1.0**
Replicates	1.41	1.42	1.38	1.36	1.99	1.34	0.71	0.14
	1.43	1.38	1.38	1.37	1.36	1.34	0.65	0.06
	1.40	1.39	1.37	1.36	1.40	1.35	0.68	0.10
	1.41							
	1.42							

	1.40	

Table 8. Endpoints based on initial measured concentrations in study with *Pseudokirchneriella subcapitata*

Test period	**Applicant analysis**			**eCA analysis**		
	ErC50 (mg a.i/L)	**ErC10 (mg a.i/L)**	**NOErC (mg a.i/L)**	**ErC50 (mg a.i/L)**	**ErC10 (mg a.i/L)**	**NOErC (mg a.i/L)**
0-24 h	0.419 (0.0924-0.745)	0.334 (0-22.6)	0.16	0.474	0.321	0.16
0-48 h	0.373 (0.350-0.396)	0.129 (0.107-0.150)	0.027	0.361	0.157	0.027
0-72 h	0.319 (0.264-0.374)	0.167 (0.079-0.253)	0.068	0.315	0.166	0.068
0-96 h	not determined	not determined	not determined	not determined*	not determined*	not determined*

* *Not calculated a*s the criterion 2 of OECD 201 guideline for 0-96 hr period was not met. For this reason 0-96 hour period should not be considered as relevant for determination of endpoints.

According to results presented in table 8 the period 48h was identified as the most sensitive for MBIT. ErC_{10} and NOErC values were during this period the lowest in both analysis provided by eCA and Applicant. Due to fast disappearance of MBIT final endpoints should be based on mean measured concentration.

Endpoint agreed at BPC- WG IV/2016 Meeting:

NOE_rC (48h) = 0.012 mg/L (based on TWA approach)

E_rC_{10} (48h) = 0.09 mg/L (based on TWA approach)

E_rC_{50} (48h) = 0.24 mg/L (based on TWA approach)

AF = 10

According to WG IV/2016 agreement it was also considered that NOEC (of 0.012 mg/L) is lower than ErC_{10} (of 0.09 mg/L) and thus it is more representative endpoint for MBIT:
PNECaq = 0.0012 mg a.i./L

Study on *Skeletonema costatum, Softcheck, 2009*

Validity criteria

According to OECD 201 guideline (adopted in 2006, with Annex 5 corrected in 2011) the following criteria should be in test on algae met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.
- The mean coefficient of variation for section-by-section growth rates (day 0-1, 1-2, 2-3 for 72-hour tests) in the control cultures must not exceed 35%. This criterion applies to the mean value of coefficient of variation calculated for replicate control cultures.
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures must not exceed 10% in tests on other species than *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus.*

Table 9. Cell density data in study with *Skeletonema costatum*

Cells density (cells/mL)					
Control	**0h**	**24h**	**48h**	**72h**	**96h**
A	77000	162500	502500	867500	1417500
B	77000	305000	555000	952500	1360000
C	77000	125000	435000	940000	1202500
Mean	77000	197500	497500	920000	1326667
Solvent control[1]	**0h**	**24h**	**48h**	**72h**	**96h**
A	77000	207500	520000	1262500	1683300
B	77000	77500	535000	955000	1716700
C	77000	107500	427500	1262500	1790000
D	77000	142500	510000	1497500	1940000
E	77000	132500	482500	1245000	1312500
F	77000	127500	545000	1372500	2070000
Mean	77000	132500	503333	1265833	1752083

According to results presented in Table 9 the biomass in the control cultures increased exponentially by a factor of 12 within 72 h period which is less than 16 required by OECD 201 guideline. However, in the guideline it is also stated that this criterion may not be met when species that grow slower than those listed in Annex 2 are used. In this case, the test period should be extended to obtain at least a 16-fold growth in control cultures. This was done in study with *Skeletonema costatum*– test was carried on for 96 hours and after this period the biomass in the control cultures increased exponentially by a factor of 17. Additionally, this validity criterion should be supplemented by requirements given in OPPTS 850.5400 guideline, according to

[1] Solvent control contained acetone. MBIT in this study was also dissolved in acetone.

which 96h cell density in the control should be approximately $150*10^4$ cells/mL, what was met.

The first criterion of OECD 201 guideline was met in solvent control cultures, where the biomass increased exponentially by a factor of 18 and 23 within 72-hour and 96-hour period, respectively.

According to results presented in Table 10 the second OECD 201 criterion in control cultures was not met. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3) in a control cultures was 46% thus it exceed the trigger value of 35%. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3 and 3-4) in a control cultures was 55%, thus it also exceed the trigger value of 35%.

What more important for this study, the second OECD 201 criterion in solvent control cultures was also not met. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3) in a control cultures was 51% thus it exceed the trigger value of 35%. Mean coefficient of variation for section-by-section specific growth rates (0-1, 1-2, 2-3 and 3-4) in a control cultures was 67%, thus it also exceed the trigger value of 35%.

In addition it should be also pointed that validity criteria were not fulfilled for 0-1 and 1-2 days –the mean CV was above 35% (49% in a control cultures and 68% in a solvent control cultures).

According to results presented in Table 11 the third OECD 201 criterion was met in controls. During the whole 72 hr and 96 hr period the coefficient of variation of average specific growth rates in replicate control cultures did not exceed 10% - it was 2 and 3%, respectively.

Criterion was also met in solvent controls. During the whole 72 hr and 96 hr period the coefficient of variation of average specific growth rates in replicate control cultures did not exceed 10% - it was 5% during both periods.

In addition it could be indicated that during the 48 hr period the coefficient of variation of average specific growth rates in replicate control cultures was also low (7 an 5% in control and solvent control replicates) (Table 12).

High differences among control replicates were however identified during the first 24 hr period (53 and 65% in control and solvent control replicates). Consequently, NOEC values seem to be not relevant for this test and thus EC_{10} should determined additionally.

As not all criteria (especially the second) were met in study with *Skeletonema costatum* the reliability of the study was decreased to RI of 3. Further assessment of study is however presented in this paper for the sake of completeness.

Table 10. Average growth rates and coefficient of variations of section-by-section growth rate in study with *Skeletonema costatum* calculated by eCA on the basis of cells density data

Average growth rate day by day					**(0-1;1-2)**			**(0-1;1-2;2-3)**			**(0-1;1-2;2-3;3-4)**		
Control	**0-24**	**24-48**	**48-72**	**72-96**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**
A	0.75	1.13	0.55	0.49	0.94	0.27	0.29	0.81	0.30	0.37	0.73	0.29	0.40
B	1.38	0.60	0.54	0.36	0.99	0.55	0.56	0.84	0.47	0.56	0.72	0.45	0.63
C	0.48	1.25	0.77	0.25	0.87	0.54	0.62	0.83	0.39	0.46	0.69	0.43	0.63
					mean		**0.49**	**mean**		**0.46**	**mean**		**0.55**
Solvent control	**0-24**	**24-48**	**48-72**	**72-96**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**
A	0.99	0.92	0.89	0.29	0.96	0.05	0.05	0.93	0.05	0.06	0.77	0.33	0.42
B	0.01	1.93	0.58	0.59	0.97	1.36	1.40	0.84	0.99	1.18	0.78	0.82	1.05
C	0.33	1.38	1.08	0.35	0.86	0.74	0.86	0.93	0.54	0.58	0.79	0.53	0.67
D	0.62	1.28	1.08	0.26	0.95	0.47	0.49	0.99	0.34	0.34	0.81	0.46	0.57
E	0.54	1.29	0.95	0.05	0.92	0.53	0.58	0.93	0.38	0.40	0.71	0.53	0.75
F	0.50	1.45	0.92	0.41	0.98	0.67	0.69	0.96	0.48	0.49	0.82	0.48	0.58
					mean		**0.68**	**mean**		**0.51**	**mean**		**0.67**

Table 11. Average growth rates and coefficient of variations of growth rate during whole test period in study with *Skeletonema costatum* calculated by eCA (PL)

Average growth rate during test period				
Control	**0-24h**	**0-48h**	**0-72h**	**0-96h**
A	0.75	0.94	0.81	0.73
B	1.38	0.99	0.84	0.72
C	0.48	0.87	0.83	0.69
mean	0.87	0.93	0.83	0.71
SD	**0.46**	0.06	0.02	0.02
CV	**0.53**	**0.07**	**0.02**	**0.03**
Solvent control	**0-24h**	**0-48h**	**0-72h**	**0-96h**
A	0.99	0.96	0.93	0.77
B	0.01	0.97	0.84	0.78
C	0.33	0.86	0.93	0.79
D	0.62	0.95	0.99	0.81
E	0.54	0.92	0.93	0.71
F	0.50	0.98	0.96	0.82
mean	0.50	0.94	0.93	0.78
SD	**0.32**	0.04	0.05	0.04
CV	**0.65**	**0.05**	**0.05**	**0.05**

Actual concentrations of MBIT in study with *Skeletonema costatum*

Concentration of MBIT in study with *Skeletonema costatum* was measured everyday (Table 12).

Table 12. Actual concentrations of MBIT in study with *Skeletonema costatum*

Concentration of MBIT					
Nominal (mg a.i./L)	**Measured (mg a.i./L)**				
	0 h	**24 h**	**48 h**	**72 h**	**96 h**
Control	<LOQ*	<LOQ**	<LOQ**	<LOQ**	<LOQ***
Solvent control	<LOQ*	<LOQ**	<LOQ**	<LOQ**	<LOQ***
0.063	0.072 (114)	<LOQ**	<LOQ**	<LOQ**	<LOQ**
0.13	0.14 (108)	<LOQ**	<LOQ**	<LOQ**	<LOQ**
0.25	0.26 (104)	<LOQ**	<LOQ**	<LOQ**	<LOQ**
0.50	0.48 (96)	<LOQ**	<LOQ**	<LOQ**	<LOQ***
1.00	1.0 (100)	0.47 (47)	0.27 (27)	<LOQ****	<LOQ****

values in brackets represent percentage of nominal concentration
LOQ* = 0.054 mg a.i./L; LOQ** = 0.050 mg a.i./L; LOQ*** = 0.049 mg a.i./L, LOQ***=0.099 mg a.i./L

Initial measured concentrations of test substance represented 96-114% of nominal concentrations.
During study this concentration declined in all test systems and was not maintained at >80% of nominal concentrations during the test. Analysis of samples collected at 72 and 96 hours showed that concentration of MBIT decreased to <LOQ all test concentrations.
For this reason the mean measured concentrations were also calculated (please refer to Table 13).

Table 13. Concentrations of MBIT in study with *Skeletonema costatum*

Initial measured concentration (mg a.i./L)	Concentration measured at (mg a.i./L)				k (1/h)	Concentration calculated at each time (mg a.i./L)				Mean measured concentrations (TWA approach) (mg a.i./L)			
	24h	48h	72h	96h		24h	48h	72h	96h	24h	48h	72h	96h
0.072	0.0250*	0.0250*	0.0250*	0.0250*	0.01	0.055	0.042	0.033	0.025	0.010	0.009	0.008	0.007
0.14	0.0250*	0.0250*	0.0250*	0.0250*	0.02	0.091	0.059	0.038	0.025	0.015	0.012	0.010	0.009
0.26	0.0250*	0.0250*	0.0250*	0.0250*	0.02	0.145	0.000	0.255	0.025	0.018	0.014	0.011	0.009
0.48	0.0250*	0.0250*	0.0250*	0.0250*	0.02	0.271	0.153	0.087	0.27	0.018	0.014	0.011	0.009
1.00	0.47	0.27	0.0495*	0.0495*	0.03	0.47	0.22	0.105	0.0495	0.022	0.016	0.012	0.010

* concentration expressed as LOQ/2

The most sensitive period in study with *Skeletonema costatum*

To identify the most sensitive period E_rC_{50} and E_rC_{10} values were calculated on the basis of initial measured concentrations by using Hill model in Regtox.EV7.06 software. NOE_rC values have been derived from Dunett`s test (5%).

Table 14. Cell density data in study with *Skeletonema costatum*

Cells density (cells/mL)					
Control	**0h**	**24h**	**48h**	**72h**	**96h**
A	77000	162500	502500	867500	1417500
B	77000	305000	555000	952500	1360000
C	77000	125000	435000	940000	1202500
Solvent control	**0h**	**24h**	**48h**	**72h**	**96h**
A	77000	207500	520000	1262500	1683300
B	77000	77500	535000	955000	1716700
C	77000	107500	427500	1262500	1790000
D	77000	142500	510000	1497500	1940000
E	77000	132500	482500	1245000	1312500
F	77000	127500	545000	1372500	2070000
0.072	0h	24h	48h	72h	96h
A	77000	220000	542500	1492500	1773300
B	77000	107500	570000	1477500	1773300
C	77000	187500	492500	1275000	1873300
0.14	0h	24h	48h	72h	96h
A	77000	107500	445000	1455000	1600000
B	77000	100000	717500	1442500	1593300
C	77000	125000	707500	1205000	2030000
0.26	0h	24h	48h	72h	96h
A	77000	112500	602500	1255000	2010000
B	77000	192500	620000	1417500	2000000
C	77000	170000	585000	1357500	1793300
0.48	0h	24h	48h	72h	96h
A	77000	112500	297500	1195000	1600000
B	77000	137500	435000	1227500	1350000
C	77000	122500	425000	1242500	1603300
1.00	0h	24h	48h	72h	96h
A	77000	42500	45000	335000	610000
B	77000	20000	27500	15000	25000
C	77000	7500	5000	0	7500

Table 15. Growth rates during different time periods in study with *Skeletonema costatum* calculated by eCA on the basis of cells density data

0-24 h						
Initial measured conc. (mg a.i./L)	**0**	**0.0072**	**0.14**	**0.26**	**0.48**	**1.0**
Replicates	0.99	1.05	0.33	0.38	0.38	-0.59
	0.01	0.33	0.26	0.92	0.58	-1.35
	0.33	0.89	0.48	0.79	0.46	-2.33
	0.62					
	0.54					
	0.50					
0-48 h						
Initial measured conc. (mg a.i./L	**0**	**0.0072**	**0.14**	**0.26**	**0.48**	**1.0**
Replicates	0.96	0.98	0.88	1.03	0.68	-0.27
	0.97	1.00	1.12	1.04	0.87	-0.51
	0.86	0.93	1.11	1.01	0.85	-1.37
	0.95					
	0.92					
	0.98					
0-72 h						
Initial measured conc. (mg a.i./L)	**0**	**0.0072**	**0.14**	**0.26**	**0.48**	**1.0**
Replicates	0.93	0.99	0.98	0.93	0.91	0.49
	0.84	0.98	0.98	0.97	0.92	-0.55
	0.93	0.94	0.92	0.96	0.93	0.00
	0.99					
	0.93					
	0.96					
0-96 h						
Initial measured conc. (mg a.i./L)	**0**	**0.0072**	**0.14**	**0.26**	**0.48**	**1.0**
Replicates	0.77	0.78	0.76	0.82	0.76	0.52
	0.78	0.78	0.76	0.81	0.72	-0.28
	0.79	0.80	0.82	0.79	0.76	-0.58
	0.81					
	0.71					
	0.82					

Table 16. Endpoints based on initial measured concentrations in study with *Skeletonema costatum*

Test period	Applicant analysis			eCA analysis		
	ErC50 (mg a.i/L)	**ErC10 (mg a.i/L)**	**NOErC (mg a.i/L)**	**ErC50 (mg a.i/L)**	**ErC10 (mg a.i/L)**	**NOErC (mg a.i/L)**
0-24 h	0.865 (NC)	0.654 (NC)	0.48	0.903	0.662	0.48
0-48 h	0.747 (0.351->1.0)	0.512 (NC)	0.48	0.927	0.597	0.48
0-72 h	0.774 (0.410->1.0)	0.621 (0.329-	0.48	0.842	0.634	0.48
0-96 h	not determined	not determined	0.48	1.305	0.719	0.48

According to results presented in Table 16 E_rC50 and E_rC10 values given by Applicant the most sensitive period would be 48 hours. According to eCA (PL) E_rC10 results the most sensitive period is also 48 hours. Due to fast disappearance of MBIT final endpoint should be based on mean measured concentration. However as not all validity criteria in study with *Skeletonema costatum* were met endpoints from this study should not be taken into further consideration.

Annex 2:

IUCLID file.