Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

4,4'-methylenedimorpholine; [MBM]

EC Number: 227-062-3
CAS Number: 5625-90-1

CLH-O-0000001412-86-94/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

4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 4-(morpholin-4-ylmethyl)morpholine

EC Number: **227-062-3**

CAS Number: **5625-90-1**

Index Number:

Contact details for dossier submitter:

Umweltbundesamt GmbH

on behalf of

AT Competent Authority

Federal Ministry of Agriculture, Forestry, Environment and Water Management

Version number: 2 **Date: 15 September 2014**

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Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name[#]:	***4-(morpholin-4-ylmethyl)morpholine***
EC number:	***227-062-3***
CAS number:	***5625-90-1***
Annex VI Index number:	***Not available***
Degree of purity:	*92.1 – 96.2 % w/w*
Impurities:	*See Doc IIA confidential**

*) Doc. II-A confidential refers to the draft Competent Authority Report which has been attached in IUCLID section 13
 # IUPAC name

Common names and synonyma are: N,N-Methylenebismorpholine, 4,4'-Methylenedimorpholine, Dimorpholinomethane, MBM

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation (including criteria according to 2nd ATP of CLP)
Current entry in Annex VI, CLP Regulation	No entry
Current proposal for consideration by RAC	Skin Corr. 1B, H314: Causes severe skin burns and eye damage Skin Sens. 1, H317: May cause an allergic skin reaction, SCL: 1.2% Carc. 1B, H350: May cause cancer Muta. 2, H341: Suspected of causing genetic defects
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Corr. 1B, H314: Causes severe skin burns and eye damage Skin Sens. 1, H317: May cause an allergic skin reaction, SCL: 1.2% Carc. 1B, H350: May cause cancer Muta 2, H341: Suspected of causing genetic defects

Please find below the harmonized classification of the hydrolysis products formaldehyde (CAS Number: 50-00-0) and Morpholine (CAS Number: 110-91-8) according to the Committee for Risk Assessment RAC (2012)[1] and the CLP Regulation (EC) No. 1272/2008[2], respectively.

According to the ECHA (2010)[3] a proposal for revision and/or removal of an entry should only include information related to those hazard classes and/or differentiations which are either not yet covered by the existing entry or need to be revised based on the information available. Because none of the above mentioned is applicable to formaldehyde and 2-hydroxypropylamine this CLH-Report focused on information concerning the reaction product of Paraformaldehyde and Morpholine (ratio 1:2).

[1] http://echa.europa.eu/documents/10162/254a73cf-ff8d-4bf4-95d1-109f13ef0f5a 2013-12-12

[2] http://echa.europa.eu/de/regulations/clp/legislation 2013-12-12

[3] ECHA (2010): Guidance on the preparation of CLH dossiers http://echa.europa.eu/documents/10162/13626/clh_en.pdf 2013-12-13

Table 2b: The current Annex VI entry and harmonised classification of the hydrolysis products formaldehyde and Morpholine

	CLP Regulation (including criteria according to 2nd ATP of CLP)
Formaldehyde	
Current opinion by RAC	Carc. 1B H350 Muta. 2 H341 Acute Tox. 3* H301 Acute Tox. 3* H311 Acute Tox. 3* H331 **Skin Corr. 1B H314** **Skin Sens. 1 H317** Specific Conc. Limits: * Skin Corr.1B; H314: C ≥ 25 % Skin Irrit. 2; H315: 5 % ≤ C < 25 % Eye Irrit. 2; H319: 5 % ≤ C < 25 % STOT SE 3; H335: C ≥ 5 % Skin Sens. 1; H317: C ≥ 0.2 %
Morpholine	
Current entry in Annex VI, CLP Regulation	Flam.Liq.3 H226 Acute Tox. 4 H302 Acute Tox. 4 H312 Skin Corr. 1B H314 Acute Tox. 4 H332

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

Table 3: Proposed classification according to the CLP Regulation (including criteria according to 2nd ATP of CLP)

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	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	n.a.	n.a.	currently not classified	data lacking
2.3.	Flammable aerosols	n.a.	n.a.	currently not classified	data lacking
2.4.	Oxidising gases	n.a.	n.a.	currently not classified	data lacking

2.5.	Gases under pressure	n.a.	n.a.	currently not classified	data lacking
2.6.	Flammable liquids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification data lacking
2.7.	Flammable solids	n.a.	n.a.	currently not classified	data lacking
2.8.	Self-reactive substances and mixtures	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	n.a.	n.a.	currently not classified	data lacking
2.10.	Pyrophoric solids	n.a.	n.a.	currently not classified	data lacking
2.11.	Self-heating substances and mixtures	n.a.	n.a.	currently not classified	data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	n.a.	n.a.	currently not classified	data lacking
2.15.	Organic peroxides	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	n.a.	n.a.	currently not classified	data lacking
3.1.	Acute toxicity - oral	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin Corr. 1B, H314: Causes severe skin burns and eye damage	n.a.	currently not classified	n.a.
3.3.	Serious eye damage / eye irritation	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	n.a.	n.a.	currently not classified	data lacking

3.4.	Skin sensitisation	Skin Sens. 1, H317: May cause an allergic skin reaction	1.2%	currently not classified	n.a.
3.5.	Germ cell mutagenicity	Muta 2, H341: Suspected of causing genetic defects	n.a.	currently not classified	n.a.
3.6.	Carcinogenicity	Carc. 1B, H350: May cause cancer	n.a.	currently not classified	n.a.
3.7.	Reproductive toxicity	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment			currently not classified	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer			currently not classified	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:

GHS Pictograms

Signal word: Danger

Hazard statements:
H314: Causes severe skin burns and eye damage
H317: May cause an allergic skin reaction
H350: May cause cancer
H341: Suspected of causing genetic defects

Precautionary statements:
P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P281: Use personal protective equipment as required
P260: Do not breathe mist/vapours/ spray.
P264: Wash ... thoroughly after handling.
P301 + P330 + P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 +P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
P363: Wash contaminated clothing before reuse.
P310: Immediately call a POISON CENTER or doctor/physician.
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
P405: Store locked up.
P501: Dispose of contents/container to …

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There is no current classification according to Annex I of Council Directive 67/548/EEC.

There is also no current classification according to Table 3.1 of Annex VI of Regulation (EC) No 1272/2008.

2.2 Short summary of the scientific justification for the CLH proposal

Human Toxicology:

By contact with biological tissues and media and with aqueous dilution 4-(morpholin-4-ylmethyl)morpholine (MBM) hydrolyses to formaldehyde and Morpholine. It is assumed that the toxicity of MBM is related to the formaldehyde release.

Formaldehyde is corrosive and also for undiluted MBM standard in vivo rabbit data are available that indicate irreversible tissue damage. Consequently MBM is proposed for classification in Cat 1B, H314 – Causes severe skin burn and eye damage~~. No sub-categorization is possible, since only the 4 hour exposure interval was tested.~~

Formaldehyde is a well-known human skin sensitizer. In a guinea pig maximization test according to current guidelines no sensitizing effects of MBM were observed. However the study was considered as inconclusive due to low irritation rates with intradermal induction and no irritation with topical induction. Consequently MBM is proposed to be classified for skin sensitization based on the mechanistic considerations of total releasable amount of formaldehyde upon contact with biological media and read across of the sensitizing property of formaldehyde. Also the specific classification limit of formaldehyde (0.2%) was read across on a molar basis (factor 6.2) and proposed as 1.2%. Alternatively MBM may not be classified for skin sensitization based on considering just the amount of free formaldehyde in MBM. Supportive arguments for both options are provided in the specific chapter on skin sensitization.

Formaldehyde is classified as Carcinogen Cat 1B (via inhalation) and Mutagenicity Cat 2 on the basis of available animal and human data. No carcinogenicity data are available for MBM, but mutagenicity data are comparable with formaldehyde. MBM is proposed to be classified for carcinogenicity cat 1B and mutagenicity cat 2 based on the mechanistic considerations of total releasable amount of formaldehyde upon contact with biological media and read across of the carcinogenic and mutagenic property of formaldehyde. Alternatively MBM may not be classified for carcinogenicity and mutagenicity based on considering just the amount of free formaldehyde in MBM. Supportive arguments for both options are provided in the specific chapter on carcinogenicity.

Environment:

MBM hydrolyses rapidly (<< 1day) to formaldehyde and morpholine in the aquatic environment. Therefore in addition to the data on MBM itself also data on the hydrolysis products formaldehyde and morpholine were considered for classification.

Acute Category:

All available acute $L(E)C_{50}$ values for MBM as well as for the hydrolysis products formaldehyde and morpholine are >1 mg/L, therefore no classification is needed for MBM.

Chronic Categories:

For MBM 2 long-term NOECs are available for crustacean and algae, which are both >1 mg/L. For fish an acute LC_{50} >100 mg/L is available and MBM is readily biodegradable; additionally a calculated log K_{ow} of -1.53 and a calculated BCF of 1.41 L/kg are available. On the basis of these data no classification for any of the chronic categories is needed for MBM.

There is only one reliable chronic NOEC value of >1 mg/L available for formaldehyde from crustacean. For fish and algae EC_{50} values >1 mg/L are available, which in combination with ready biodegradability, a measured log K_{ow} of 0.35 and a calculated BCF_{fish} of 0.396 L/kg doesn't lead to any classification.

Morpholine shows a chronic NOEC for algae of >1 mg/L. For fish and crustacean there are acute $L(E)C_{50}$ ≥100 mg/L available. In addition morpholine is readily biodegradable; it has a measured log K_{ow} =-0.86 and a measured BCF <2.8 L/kg. Again these data don't lead to any classification for morpholine.

Hazards to the ozone layer:

On the basis of low vapor pressure, low Henrys Law constants and rapid degradation through reaction with hydroxyl radicals for MBM as well as for its hydrolysis products there are no indications for danger to the ozone layer.

Conclusion:

No classification for hazards to the aquatic environment and to the ozone layer is proposed for MBM, since neither the available data on MBM itself, nor the data on its hydrolysis products fulfill the criteria.

2.3 Current harmonised classification and labelling

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

No current classification and labelling.

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

No current classification and labelling.

2.4 Current self-classification and labelling

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

The applicant self-classified MBM based on DSD criteria. The main self-classification according to CLP listed in the C&L inventory[4] is depicted below:

Acute Tox. 4	H302: Harmful if swallowed
Skin Corr. 1C	H314: Causes severe skin burns and eye damage
Eye Dam. 1	H318: Causes serious eye damage

[4] http://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/cl-inventory/view-notification-summary/2355 (accessed on: 14.07.2014)

Skin Sens. 1	H317: May cause an allergic skin reaction
Skin Irrit. 2	H315: Causes skin irritation
Eye Irrit. 2	H319: Causes serious eye irritation
Skin Corr. 1B	H314: Causes severe skin burns and eye damage
Aquatic Chronic 3	H412: Harmful to aquatic life with long lasting effects
Met. Corr. 1	H290: Maybe corrosive to metals
STOT SE 3	H335: May cause respiratory irritation

2.4.2 Current self-classification and labelling based on DSD criteria

Classification	By the manufacturer
Class of danger	C (corrosive)
R phrases	R22: Harmful if swallowed R34: Causes burns R52: Harmful to aquatic organisms/38
S phrases	S26, S36/39, S37, S45, S61

RAC general comment
On contact with biological tissues and media and with dilute aqueous media, 4-(morpholin-4-ylmethyl)morpholine (MBM) hydrolyses to formaldehyde and morpholine. It is assumed that the toxicity of MBM is related to the released formaldehyde. Where data on MBM were not available, data from the hydrolysis products was considered. The maximum (calculated) 'releasable' formaldehyde per molecule of MBM is 16.7% w/w.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Biocides: No need for justification.

Also conclusion for non-classification for the various endpoints is of utmost importance for European harmonisation. RMS proposals for classification and non-classification were not discussed in detail within the European Biocides Technical Meetings

Part B.

SCIENTIFIC EVALUATION OF THE DATA

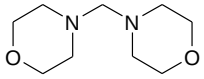
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	227-062-3
EC name:	N,N'-methylenebismorpholine
CAS number (EC inventory):	Not available
CAS number:	5625-90-1
CAS name:	N,N’-Methylenebismorpholine
IUPAC name:	4-(morpholin-4-ylmethyl)morpholine
CLP Annex VI Index number:	Not available
Molecular formula:	$C_9H_{18}N_2O_2$
Molecular weight range:	186.26 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
N,N'-methylenebismorpholine	94.2 % w/w	92.1 – 96.2 % w/w	--

For the constituent N,N'-methylenebismorpholine the concentration range is derived as mean value ± 3 x standard deviation.

The manufacturer has requested that all impurities remain confidential since it may provide an indication on the possible method of manufacturing. Information on impurities is provided in the confidential IUCLID section 1.2 (Composition) and in Doc. II-A confidential of the draft Competent Authority Report attached to IUCLID section 13.

Only impurities > 0.1 % w/w have been identified. Based on a 5-batch analysis, up to 98.6 % w/w of the substance have been identified and quantified. See Doc IIA and Doc IIA confidential of the draft Competent Authority Report, For the remaining impurities no information is available.

The substance does not contain any additives.

Current Annex VI entry: No current Annex VI entry.

1.2.1 Composition of test material

As the substance as manufactured is used as biocidal product several studies use the trade names as denomination of the test substance instead of the chemical name. Known trade names which refer to the same substance as described in chapter 1.2 are CONTRAM™ ST-1 and BIOZID ST-1.

The test materials used were in compliance with the specifications as lied down by the 5-batch analysis mentioned above. For details of the specification, which has been claimed confidential by the manufacturer, see Doc. II-A confidential of the draft Competent Authority Report attached to IUCLID section 13.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Method	Purity/Specification	Results	Reference
Melting point	EC method A.1	Identification number: OS 157340 Purity: 98%w/w	18 - 21 ± 0.5°C	Doc. III-A 3; Study A 3.1.1/
Boiling point	EC method A.2	Identification number: OS 157340 Purity: 98% w/w	266.4 ± 0.5°C	Doc. III-A 3; Study A 3.1.1
Relative density	EC method A.3	Identification number: OS 157340 Purity: 98% w/w	Relative density: 1.05 (20°C)	Doc. III-A 3; Study A 3.1.1
	DIN 517757D	Contram ST-1 Batch no.100500234 Purity: min.92.1%w/w (a.s. as manufactured)	Density: 1.0647 g/cm^3 at 20°C	Doc. III-A 3; Study A 3.1.3
Vapour	calculated with	n.a.	0.625 Pa (25°C, calculated with	Doc. III-A 3;

Property	Method	Purity/Specification	Results	Reference
pressure	EpiSuite 3.12; calculated with EUSES		EpiSuite 3.12); 0.443 Pa (20°C, calculated with EUSES)	Study A 3.2b
Henry´s Law Constant	Epi Suite 3.12 HENRYWIN v3.10	n.a.	2.72x10 $^{-5}$ Pa x m^3/mol (25°C, calculated with EpiSuite 3.12)	Doc. III-A 3; Study A 3.2b
Physical state	Visual inspection	n.a.	Liquid	Doc. III-A 3; Study A 3.1.1
Colour	Visual inspection	n.a.	Extremely pale yellow	Doc. III-A 3; Study A 3.1.1.
Odour	Olfactory inspection	n.a.	Slightly amine like	Doc. III-A 3; Company Statement
Absorption spectra: UV/VIS	Spectralphoto-metric determination	CONTRAM™ ST-1, Lot Number 100428662 Purity: min.92.1%w/w (a.s. as manufactured)	UV/VIS spectrum is consistent with the proposed structure of MBM. There are no absorption maxima above 290 nm.	Doc. III-A 3; Study A 3.4/01
Absorption spectra: IR	Spectralphoto-metric determination	CONTRAM™ ST-1, Lot Number 100500234 Purity: min.92.1%w/w (a.s. as manufactured)	IR spectrum is consistent with the proposed structure of MBM.	Doc. III-A 3; Study A 3.4/02
Absorption spectra: NMR	^{1}H-NMR ^{13}C-NMR	N,N-Methylene bismorpholine Purity: min.92.1%w/w (a.s. as manufactured)	^{1}H- NMR spectrum and ^{13}C- NMR spectrum is consistent with the proposed structure of MBM.	Doc. III-A 3; Study A 3.4/03, Doc. III-A 3; Study A 3.4/05

Table 9: Summary of physico-chemical properties (continued)

Property	Method	Purity/Specification	Results	Reference
Absorption spectra: **MS**	EI-MS	Contram ST-1 Batch no.100500234 Purity: min.92.1%w/w (a.s. as manufactured)	MS spectrum is consistent with the proposed structure of MBM.	Doc. III-A 3; Study A 3.4/04
Water solubility	EC method A.6	Identification number**:** OS 157340 Purity: 98% w/w	Test substance is hydrolysable; miscible in all proportions temperature: 10.0 – 30.0 ± 0.5°C pH: 5 - 9	Doc. III-A 3; Study A 3.1.1
Dissociation constant	Estimation by using QSAR	n.a.	Calculated: pKa = 7.39 and 2.98 at 20°C Test substance is hydrolysable, therefore determination of the pKa is not possible.	Doc. III-A 3; Study A 3.6a
Dissociation constant	DIN 51369 Method:	Contram ST-1 Batch no.100500234 Purity: min.92.1%w/w (a.s. as manufactured)	1. 0% CONTRAMTM ST-1 in dist. Water pH = 10.48 at 20°C	Doc. III-A 3; Study A 3.6b
Solubility in organic solvents, including the effect of temperature on solubility	Hach Method 8195	Contram ST-1 lot number 100430887 Purity: min.92.1%w/w (a.s. as manufactured)	Solubility in n-heptane: 2000 – 2500 mg/L (20.5°C)	Doc. III-A 3; Study A 3.7a
	visual inspection for turbidity or phase separation	Contram ST-1 lot number 100480548 Purity: min.92.1%w/w (a.s. as manufactured)	Completely miscible in DMSO, Toluene, Ethanol, n-Octanol and Acetone Partially soluble in Cyclohexane (Concentrations tested: 5000, 2500, 1000, and 500 mg/mL at 21-23 °C)	Doc. III-A 3; Study A 3.7b
Stability in organic solvents used in b.p. and identity of relevant breakdown products	Justification	n.a.	The substance and the biocidal products are solely handled and marketed as aqueous solution which contains no organic solvents.	Doc. III-A 3; Justification
Partition coefficient n-octanol/water	EC method A.8	Identification number**:** OS 157340 Purity: 98% w/w	log Pow = < 0.3 pH : 5, 7, 9 at 30°C (Test substance is hydrolysable)	Doc. III-A 3; Study A 3.1.1
	Epi Suite 3.12 KOWWIN v1.67	n.a.	Calculation: log Pow = -1.53 (EpiSuite)	Doc. III-A 3; Study A 3.2
Thermal stability identity of relevant breackdown products	Differential Scanning Calorimetry (DSC)	Contram ST-1 BC:6005/100500234 Purity: min.92.1%w/w (a.s. as manufactured)	Substance can be safely handled up to 115°C	Doc. III-A 3; Study A 3.10

Table 9: Summary of physico-chemical properties (continued)

Property	Method	Purity/Specification	Results	Reference
Flammability, including autoflammability and identity of combustion products	Justification	n.a.	Melting point is below 100°C. Therefore, determination of flashpoint is sufficient for the testsubstance.	Doc. III-A 3; Justification
Flash-point	ASTM / OSHA 1910.106, open-cup	Contram ST-1 Charge no:100550330 Purity: min.92.1%w/w (a.s. as manufactured)	115 °C (open cup method)	Doc. III-A 3; Study A 3.12
Surface tension	Justification	n.a.	Not applicable due to hydrolysis in aqueous solution	Doc. III-A 3; Justification
Viscosity	DIN 51562 part I	Contram ST-1 Charge no:100500234 Purity: min.92.1%w/w (a.s. as manufactured)	16 mPa.s (20°C)	Doc. III-A 3; Study A 3.14
Explosive properties	Justification	n.a.	There is no structural alert for explosive properties.	Doc. III-A 3; Justification
Oxidising properties	Justification	n.a.	There is no structural alert for oxidizing properties.	Doc. III-A 3; Justification
Reactivity towards container material	Company Statement	n.a.	The biocidal product is packed and stored in LDPE containers or in steel barrels or containers coated with LDPE. Long-time experience shows that these materials are suitable for storage and transport of the biocide.	Doc. III-A 3; Company Statement
Granulometry	no data available	no data available	no data available	no data available

2 MANUFACTURE AND USES

2.1 Manufacture

Biocides: Does not need to be specified for the CLH proposal.

2.2 Identified uses

In-can preservative, product type 6

Metal-working fluid, product type 13

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Property	Purity/Specification	Results	Reference
Thermal stability identity of relevant breakdown products	Contram ST-1 BC:6005/100500234 Purity: min.92.1%w/w (a.s. as manufactured)	Substance can be safely handled up to 115°C	Doc. III-A 3; Study A 3.10
Flammability, including autoflammability and identity of combustion products	Company Statement	Melting point is below 100°C. Therefore, determination of flashpoint is sufficient for the test substance.	Doc. III-A 3; Justification
Flash point	Contram ST-1 Charge no:100550330 Purity: min.92.1%w/w (a.s. as manufactured)	115 °C (open cup method)	Doc. III-A 3; Study A 3.12
Explosive properties	Company Statement	There is no structural alert for explosive properties.	Company Statement
Oxidizing properties	Company Statement	There is no structural alert for oxidizing properties.	Company Statement
Reactivity towards container material	Company Statement	The biocidal product is packed and stored in LDPE containers or in steel barrels or containers coated with LDPE. Long-time experience shows that these materials are suitable for storage and transport of the biocide.	Company Statement

3.1 All hazard classes

3.1.1 Summary and discussion of all hazard classes

No classification is proposed based on available data.

3.1.2 Comparison with criteria

No classification is proposed based on available data.

3.1.3 Conclusions on classification and labelling

No classification is proposed based on available data.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.1.1. Percutaneous absorption of MBM

Human skin samples were exposed for 8 h to 0.15% or 3% labelled MBM (cf. MBM – Doc III A6.2_1, Lubrizol DE 2007; OECD guideline 428). Two different 14C-labels were used: N,N'-[14C]-methylenebismorpholine (labelled methyl group) and N,N'-methylene[U-14C]-bismorpholine (labelled

morpholine). Up to 24 h after application of the labelled test substance samples were collected in receptor fluid and volatile material in carbon traps. Radioactivity was measured at termination in stratum cornea samples, remaining skin, receptor fluid, carbon trap extracts, tissues and washes. Since MBM hydrolysis to formaldehyde and morpholine the authors tested 4 different test preparations in tissue culture medium plus additives:

1) 3% MBM, mixture of radio-labelled MBM: a) 14C-label of methyl group and b) 14C-label of morpholine (detecting FA+MBM+Morpholine)
2) 0.15% MBM; mixture of radio-labelled MBM: a) 14C-label of methyl group and b) 14C-label of morpholine (detecting FA+MBM+Morpholine)
3) 0.15% MBM; MBM solely labelled at the methyl group (detecting FA+MBM)
4) 0.15% MBM; MBM solely labelled at the morpholine (detecting MBM+Morpholine)

After 24h (exposure period lasted for 8h) the percutaneous absorption of test preparation 1, 2, 3, and 4 was 61, 34, 18, and 31% of the applied dose (corrected for total recovery). The total recovery was very similar in all 4 test preparations and reached 76-79% indicating potential loss of applied radioactivity from test system. There is some evidence that dermal absorption increased with concentration comparing test preparation 1 and 2 of the same composition. These distinctions might be related to differences in hydrolysis.

Test preparation 3 showed the highest amount of radioactivity retained in the stratum corneum and lowest amount in the receptor fluid. These differences to the other test preparations might be related to the product of hydrolysis in aqueous solutions. In test preparation 3 MBM was 14C-labelled solely at the methyl group which allows detecting either MBM or hydrolysed formaldehyde. Formaldehyde obviously reacted with macromolecules of the skin especially at the outer cell layers, the stratum corneum. These chemical reactions limited further penetration through the skin and less of labelled molecules reached the receptor fluid (compared to preparation 4 in which Morpholine is labelled, means MBM + Morpholine is measured).

There is no obvious difference in dermal absorption between test preparation 2 and 4 except a slight decrease of radioactivity in stratum corneum and exposed skin and slight increase in receptor fluid which might be related to the hydrolysis product formaldehyde (binding to macromolecules in the skin tissue, additionally labelled in preparation 2).

Distribution: Specific conclusion cannot be drawn from these in vitro experiments but there is some indication that after hydrolysis morpholine will be distributed in the body (high amount measured in receptor fluid) and should be bio-available whereas formaldehyde reacts at the site of 1st contact (high amount measured in stratum corneum). This is in accord with the available data on the products of hydrolysis (see Section 4.1.2).

Even with the multiple radioactive label design chosen no quantitative estimation of the individual absorption rates of the individual components MBM, Formaldehyde and Morpholine is possible. However in June 2014 the BPC- WG concluded from the data presented below the following dermal absorption rates to be used for risk assessment:

- MBM (0.15%): 60% (from preparation 2)
- MBM (3%): 70% (from preparation 1)
- Morpholine: 50% (from preparation 4)

All toxicokinetic data in % of applied dose ±SD. Exposure period 8 h; parameters 24 h after application of the test substance

Test preparation (see 3.3.2)	1	2	3	4
Concentration of test substance	3%	0.15%	0.15%	0.15%
Site of 14C-label	Methyl group & morpholine	Methyl group & morpholine	Methyl group	Morpholine
dislodged dose after 8 h	22 ± 14	30 ± 8	38 ± 6	38 ± 8
Total dislodged dose (24 h)	24 ± 13	34 ± 6	40 ± 6	42 ± 6
Retained in stratum corneum (20 tape strips)	5.3 ± 2.1	17 ± 7.1	23 ± 5.0	13 ± 6.2
Exposed skin	10 ± 4.4	10 ± 5.0	8.0 ± 5.3	5.7 ± 3.8
Unexposed skin	3,6 ± 4.4***	4.5 ± 7.2 **	1,8 ± 5.5*	3,8 ± 5.5§
Receptor fluid and rinse	33 ± 15	11 ± 6.9	4.2 ± 2.9	15 ± 7.2
Total recovery	76 ± 5.5	78 ± 5	77 ± 2.8	79 ± 4.4
Dermal absorption = stratum corneum + exposed skin + unexposed skin + receptor fluid and rinse	52	43	37	37
Dermal absorption corrected for recovery	**68**	**56**	**48**	**47**

Dislodged dose: radioactivity in skin/cell wash, tissue swabs, pipette tips, traps and carbon filters
Unexposed skin: skin under the cell flange was cut away from exposed skin
Exposed skin: skin exposed to the radio-labelled test substance but stratum corneum removed
***: 5 out of 10 cells with high values (2.8-13%) (Presumably also exposed skin cut away), others only 0.02-1.2%;
**: in cell 6-8 (out of 10) values between 10 and 21% were measured;
*: in cell 9 (out of 10) an exceptional high value was measured;
§: 4 out of 10 cells with high values (3.2-14%).
In the absence of raw data, the eCA cannot recalculate:

- the values for the first 2 tape strips only; however from graphical representation it appears that these values are between 2% and 6%, so this represents only limited possibilities for refinement of the total absorption estimate
- the SD of the total dermal absorption estimate corrected for recovery; however considering the SD values of the individual mean values contributing to the total dermal absorption values we consider that further refinement of the total absorption estimate is not necessary

4.1.1.2. Intratracheal instillation of MBM (Doc IIIA 6.2_2, Lubrizol DE 2007a,b)

Radioactive labelled material was similar to that of the percutaneous absorption: a) 14C-label of methyl group and b) 14C-label of morpholinewere used in separate parts of the study. Dividing the study in two parts was necessary due to different requirement during follow-up of radioactive excretion:

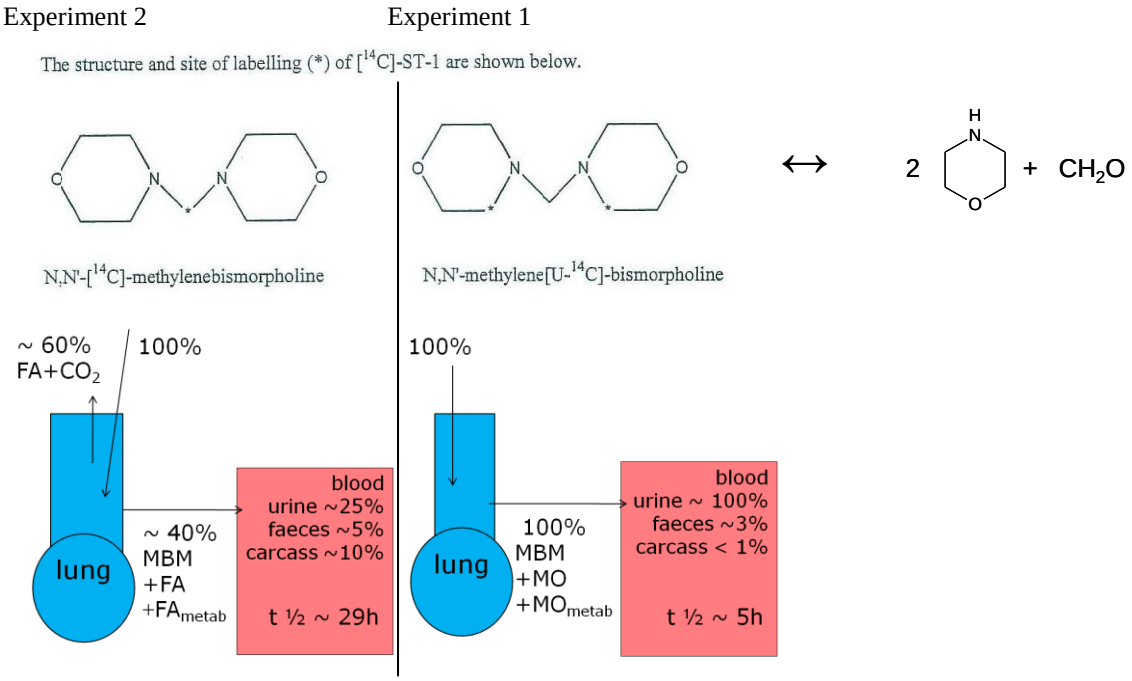
In part 1 a total of 12 Wistar rats (6 m/6 f) were dosed with N,N'-Methylenebis[U-14C]morpholine at 2.5 mg/kg bw by single intratracheal instillation. This labelling allows detection of the sum of MBM, Morpholine (MO) and some Morpholine metabolites (MOmetab, not Formaldehyde). Excretion of N,N'-Methylenebis[U-14C]morpholine via faeces and urine was studied in 4 m / 4 f rats over a period of 7 days, while blood kinetics of N,N'-Methylenebis[U-14C]morpholine were investigated in 2 m / 2 f rats over 24 hrs. The recovery of labelled compound in faeces, urine and blood was determined and at necropsy at day 7, radioactivity in organs was measured. About 100 % of the dose administered were excreted via urine during the 7 day collection period. Only marginal activities were detected in faeces accounting for 3 % of the total dose. Very low activity levels were detected in organs and tissues after 7 days and the total activity in the carcass accounted for less than 0.4 % of the dose administered at this time point. Concerning blood kinetics it was shown that the substance was rapidly absorbed after intratracheal instillation with a Cmax being

reached after 2 hrs (1st time point examined). Blood levels of N,N'-Methylenebis[U-14C]morpholine accounted for 2 % of the total dose at Tmax and declined rapidly thereafter with a blood half life (T1/2) of about 5 hrs.

In part 2 a total of 12 Wistar rats (6 m/6 f) were dosed with N,N'-[14C]Methylene-bismorpholine at 2.5 mg/kg bw by single intratracheal instillation. This labelling allows detection of the sum of MBM, Formaldehyde (FA), CO2 and Formaldehyde-metabolites (FAmetab). Expiration of N,N'-[14C]Methylene-bismorpholine (sum of FA+CO2) was studied in 2 m / 2 f rats during the 7 day sampling period. Excretion of N,N'-[14C]Methylene-bismorpholine via faeces and urine was studied in 4 m / 4 f rats over 7 days, while blood kinetics of N,N'-[14C]Methylene-bismorpholine were investigated in 2 m / 2 f rats over 24 hrs. The recovery of labelled compound in faeces, urine and blood was determined and at necropsy at day 7, radioactivity in organs was measured. It was shown that about 60% of the dose applied were expired during the 7 day sampling period (as FA+CO2) and abut 25% of the dose were recovered in urine during 7 days. Smaller amounts of radioactivity were detected in faeces, abut 5% of the dose. Notable activity levels were detected in organs and tissues after 7 days and the total activity in the carcass accounted for about 10 % of the dose administered at this time point. (All percentages are given after normalization with recovery). Blood levels of N,N'-[14C]Methylene-bismorpholine accounted for 1.3 to 2.8 % of the total dose at Tmax. Cmax was reached after 2 hrs (1st time point examined) in 3 of 4 rats and after 8 hrs in another female rat. In contrast to N,N'-Methylenebis[U-14C]morpholine the blood levels of N,N'-[14C]Methylene-bismorpholine declined slowly, resulting in long half-lives of 25 to 35 hrs.

The following figure may help to summarize the findings of the intratracheal instillation study:

Figure 4.1.-1 Intratracheal instillation study



In conclusion, percutaneous absorption varied in this in vitro study between 0.1-0.4% after 1 h, 1-2% after 2 h and 12- 43% after 24 h, depending on concentration and site of labelling.

N,N'-Methylene-bismorpholine is rapidly absorbed after intratracheal instillation. Excretion via urine of radioactivity is rapid for the morpholine-labelled MBM and for methylene-labelled MBM about 60% were expired into air. These differences in the pharmacokinetic behaviour and considering furthermore the huge differences in the half-life times of N,N'-Methylenebis[U-14C]morpholine and N,N'-[14C]Methylenebismorpholine suggest, that a large fraction of MBM is cleaved in morpholine and formaldehyde. No quantitative conclusion with regard to the individual components of the active substance (MBM, FA, MO) is possible.

4.1.1.3. Products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.1 Formaldehyde – Doc III A6.2)

In humans as well as in animals formaldehyde is an essential metabolic intermediate. Formaldehyde is absorbed and deposited after inhalation in the upper respiratory tract, the site of first contact. The physiological level of formaldehyde in the blood of humans and experimental animals is not increased after inhalation exposure due to the rapid metabolism and reactivity at the site of first entry. The rate of uptake of 14C labelled formaldehyde through human skin ex vivo was approx. 20 or 300 µg/cm2/h for 4 or 37% formaldehyde solutions, respectively, predicting 40-65 % systemic absorption from 10 µL/cm^2 applied for 8 hours under occlusion. (Loden et al. 1986).

The eMS calculated the dermal absorption in terms of flux according as follows: A mean flux of about 20 µg/cm^2 h or 300 µg/cm^2 h was estimated for a 3.7% or a 37% formaldehyde solution, respectively. According to Fick's Law (and supported by the flux estimates for 37% vs. 3.7% formaldehyde solution) it may be assumed that the flux decreases proportionally with reduced concentration. The following arguments support the use of flux estimates:

- ✓ Dermal absorption is dependent on the concentration of the substance in the product (the higher the concentration the higher the flux according to Fick´s Law) and on the exposure time. Experiments for dermal absorption usually differ in terms of concentration and exposure time from the real life exposure situation to be estimated. Flux rates estimated on the basis of Fick´s Law allow a scientifically justified extrapolation between different concentrations and exposure times. Therefore the use of flux rates may be considered for refined dermal exposure assessment.
- ✓ EFSA 2012, REACH guidance (Volume III, Part B, Guidance on regulation (EU) No 528/2012) and OECD 2011 guidance supports the use of flux rates for refining exposure estimates in the context of a triple-pack approach. Limitations of the use of flux is just indicated for the case that the amount remaining in skin is not included in the flux estimate. Furthermore the mean flux may not be adequate to estimate absorption with very short exposure times in case in the experiment initial maximal flux is much higher than the mean flux (which is not to be expected where binding to skin proteins is assumed and the amount in skin is included in the flux estimate)
- ✓ In the specific case of formaldehyde flux estimate from the Loden et al. 1986 the use of Fick´s law for extrapolating from the high concentration to the low concentration represents a conservative estimate since
    - o absorption into the superficial skin layer was included in the flux rate estimate and
    - o flux was decreased a little more than linear from the higher concentrated solution (37% formaldehyde, 300 µg/cm^2 h) to the lower concentrated solution (3.7% formaldehyde, 20 µg/cm^2 h) and the lower flux value is used to extrapolate linearly to lower concentration values (0.026% formaldehyde): The concentration of MBM in the metal working fluid is 0.15%, with 17% total releasable formaldehyde corresponding to 0.026% FA in mwf, i.e. a factor of about 145 below 3.7%. Therefore the mean flux of 20 µg/cm^2 h (mean+SD & skin+SD) for the 3.7% formaldehyde solution was divided by 145 resulting in a flux estimate of 0.15 µg/cm^2 h for the 0.026% formaldehyde solution
    - o flux rate is used just for PT13 exposure estimates with 8 hours exposure time

However the BPC-WG in June 2014 rejected the use of flux estimates. A conservative value of 65% dermal absorption rate, not corrected for concentration and time differences between experiment and real life exposure should be used.

Formaldehyde is rapidly and nearly completely absorbed from the intestinal tract after oral exposure. For risk assessment 100% absorption via all routs of exposure has to be assumed, though predominantly reaction products and metabolites of formaldehyde will be systemically available.

The oxidation of formaldehyde to formic acid catalysed by formaldehyde dehydrogenase is considered to be the main defence mechanism against the formation of covalent binding of formaldehyde to macromolecules like proteins or DNA. Formaldehyde is eliminated rapidly as formic acid in the urine or as CO_2 in the expired air or it enters the carbon pool in the body.

Morpholine (Morph-Doc III A6.2 and Morph-Doc III A6.2 Additional Information)

Since the local effects due to the high pH value predominate in dermal toxicity studies no information on systemic availability can be derived from these studies. No experimental data are available on percutaneous absorption of morpholine. However an in vitro dermal absorption study with human skin is available for MBM 0.15% with a label at the morpholine ring (sample 4). This label allows detecting the sum of integer MBM and morpholine absorbed through the skin. Assuming that MBM at 0.15% in emulsifier plus water applied to and expectedly reacting with skin is largely hydrolysed to formaldehyde and morpholine, it may be expected that most of the 31% of radioactivity absorbed through the skin represents morpholine. On the basis of this assumption there is evidence that the absorption data derived for MBM are also applicable to morpholine.

Almost complete absorption after oral application was evident in rats. With parenteral exposure Morpholine was excreted via urine in unchanged form in rats, hamsters and rabbits. In the guinea pig, however, additionally 20% of the applied dose was excreted as N-methylmorpholine-N-oxide. Other references indicate that the Morpholine ring may be cleaved by mammalian metabolism. The data suggested systemic availability of Morpholine.

Table 4.1-2 Toxicokinetics and metabolism of products of hydrolysis

Endpoint	Formaldehyde			Morpholine		
	Dermal	Inhalation	Oral	Dermal	Inhalation	Oral
Absorption	40% or 65% Human skin in vitro with 4% or 36% solution applied as 10 µL/cm^2 for 8 hours	almost complete	almost complete	No specific data (corrosive) Some evidence that MBM absorption data also applicable to morpholine	No data	Almost complete in rats
Distribution	Reactivity at the site of first entry (covalent binding to proteins and DNA) and rapid oxidation to formic acid (defence mechanism)			Rapidly excreted, high amounts in stomach and intestine after oral application		
Main metabolites	Formic acid: a) further uptake into the carbon-1-metabolic pathway, b) cleavage to CO_2 and exhalation, c) excretion of sodium formate via urine			Excreted mainly unchanged; N-methylmorpholine-N-oxide only in guinea pig		
Excretion	Exhaled CO_2 Urine: sodium formate			Excreted unchanged via urine		

4.1.1.4. Comparison of dermal absorption studies with data on products of hydrolysis

Table 4.1-3 Comparison of dermal absorption

Endpoint	MBM	Formaldehyde	Morpholine
Dermal absorption	18-61% with 0.15% or 3% solution (and various C^{14} labels) applied as 10 µL/cm^2 for 8 hours	40% or 65% with 4% or 36% solution applied as 10 µL/cm^2 for 8 hours	No specific data Some evidence that MBM absorption data also applicable to morpholine
Comments	In vitro study human skin	In vitro study human skin	Almost complete absorption in oral studies (rat)

4.1.2 Human information

See chapter 4.1.

4.1.3 Summary and discussion on toxicokinetics

The in vitro percutaneous absorption of radiolabelled MBM through human skin is documented; dermal absorption (including hydrolysis products) depends on exposure time and concentrations, but appears overall limited. Oral exposure is supposed to be not relevant for this biocidal active substance. Moreover, in aqueous solutions and biological systems MBM hydrolyses to formaldehyde and morpholine. Therefore inhalation is assumed to occur predominantly to the hydrolysis product formaldehyde. However, to support the assumption of hydrolysis also after inhalation of MBM i.e. via aerosols in metalworking an intratracheal instillation with radiolabelled MBM has been performed. Both N,N'-Methylenebis[U-14C]morpholine as well as N,N'-[14C]Methylene-bismorpholine are rapidly absorbed after intratracheal instillation. The differences in the pharmacokinetic behaviour of N,N'-Methylenebis[U-14C]morpholine and N,N'-[14C]Methylenebismorpholine suggest, that a large fraction of ST-1 is cleaved in morpholine and formaldehyde. However no quantitative conclusion with regard to the individual components of the active substance (MBM, FA, MO) is possible (for details please see (MBM – Doc III A6.2_01 and Doc III A 6.2_02).

For formaldehyde 100% absorption via all routs of exposure has to be assumed, though predominantly reaction products and metabolites of formaldehyde will be systemically available.

The oxidation of formaldehyde to formic acid catalysed by formaldehyde dehydrogenase is considered to be the main defence mechanism against the formation of covalent binding of formaldehyde to macromolecules like proteins or DNA. Formaldehyde is eliminated rapidly as formic acid in the urine or as CO2 in the expired air or it enters the carbon pool in the body.

For morpholine there is evidence that the dermal absorption data derived for MBM are also applicable to morpholine. Almost complete absorption of morpholine after oral application was evident in rats.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral - MBM

Table 4.2-1 Summary of acute oral toxicity data for MBM

Route	Method Guideline	Species Strain Sex no/group	dose levels [mg/kg bw]	Value No. dead/total LD50/LC50 [mg/kg bw]	Remarks	Reference
Oral	Acute toxic class method OECD 423	Rat Sprague-Dawley 3 m & 3 f	200 (2% sol.) 500 (undil.) 2000 (undil.)	0/6 (m,f) 1/3 (m, in 1h), 0/3 (f) 3/3 (f, in 1-4h) 500 < LD50 < 2000 (m&f combined).	local effects in the gastro-intestinal tract; males more sensitive	Lubrizol (2000) MBM-Doc III A6.1.1

4.2.1.2 Acute toxicity: inhalation - MBM

Not available.

The implementation of such a study is scientifically unjustified. It is assumed that MBM will produce severe local effects and estimations for respective NOAECs are available for MBM and formaldehyde as hydrolysis product (justification of non-submission; cf. MBM–Doc III A6.1.2 and A6.1.3 and Doc II-A 4.3).

4.2.1.3 Acute toxicity: dermal - MBM

Not available.

The implementation of such a study is scientifically unjustified. It is assumed that MBM will produce severe local effects and estimations for respective NOAECs are available for MBM and formaldehyde as hydrolysis product (justification of non-submission; cf. MBM–Doc III A6.1.2 and A6.1.3 and Doc II-A 4.3).

4.2.1.4 Acute toxicity - Products of Hydrolysis

Formaldehyde (for summary and discussion please see Formaldehyde - Doc II A3.2 & Formaldehyde - Doc III A6.1.1-6.1.3)

The submitted database would require classification of formaldehyde according to Directive 67/548/EEC as:

"Harmful if swallowed" (R22), based on LD50 values between 640 and 800 mg/kg bw in rats,

"Toxic in contact with skin" (R24), based on a dermal LD50 of 270 mg/kg bw in rabbits, and

"Toxic by inhalation" (R23), based on LC50 values of 1 mg/L x 0.5 h and 0.6 mg/L x 4 h in rats.

According to the Regulation (EC) No 1272/2008, the available data as summarised above would require classification and labelling as follows:

Category 4, Harmful if swallowed, H302

Category 3, Toxic in contact with skin, H311

Category 2 (gases), Fatal if inhaled, H330

.

Morpholine (Morph-Doc II A4.2 & Morph-Doc III A6.1.1-3 Additional Information)

The available data indicated low acute toxicity of morpholine after oral, dermal or inhalation exposure. Predominantly local effects are reported which are due to the high pH value of morpholine; other symptoms

seem to be secondary. It is considered that the local effects are concentration dependent (lower LD50 values with undiluted morpholine).

The oral LD50 in rats varied between 1050 and 1900 mg/kg bw (limited documentations). In guinea pigs an oral LD50 of 900 mg/kg bw was observed. Acute dermal toxicity studies in the rabbit revealed a LD50 of 500 mg/kg bw (limited validity); however, death was presumable caused by the corrosive effects of morpholine. Symptoms described in acute inhalation toxicity were haemorrhages of nose, mouth and eyes as well as spasm and tremor in rats; the LC50 in male and female rats was reported to be ca. 8 mg/L (limited documentation). Similar LC50 values for mice are available (6.9(f) and 5.2. (m) mg/L).

4.1.1.5. Acute Toxicity: Comparison of MBM with products of hydrolysis

Table 3.2-2 Comparison of acute toxicity data

Endpoint	MBM	Formaldehyde	Morpholine
Acute dermal toxicity (skin irritation)	No data (corrosive)	LD50 = 270 mg/kg bw (rabbit, corrosive)	LD_{50} 500 mg/kg bw (rabbit, corrosive)
Acute inhalation toxicity	No data	LC_{50}(4h) 0.6 mg/L (rat)	LC_{50} ca. 8 mg/L (rat)
Acute oral toxicity	500 < LD_{50} < 2000 mg/kg bw (rat)	LD_{50} 100-800 mg/kg bw (rat)	LD_{50} 1050 mg/kg bw (rat)

4.2.2 Human information

Not available.

4.2.3 Summary and discussion of acute toxicity

For MBM no information on acute toxicity in humans is available. The acute toxicity of MBM after oral exposure has been investigated in a valid study on experimental animals (see table 4.2-1 above). There is evidence from pathology results in rats which died during post exposure observation period that toxicity of the undiluted test substance after oral administration is due to local effects on the mucous membranes in the gastro-intestinal tract. No effects were detected in survivors. For the undiluted MBM the LD50 for males and females is greater than 500 mg/kg bw but less than 2000 mg/kg bw. No effects were observed in rats receiving the diluted test substance (2%) at 200 mg/kg bw (cf. MBM – Doc III A6.1.1). It is considered that the LD50 is not very concentration dependent, since the major part of the local corrosive effect in the GI is expected to stem from formaldehyde-protein reactions, for which the total amount of formaldehyde/releaser applied is expectedly more important than the concentration.

No respiratory LC50 and no dermal LD50 is available for MBM. However the respiratory LC50 of formaldehyde and the dermal LD50 of formaldehyde may be read across to MBM for classification purposes.

4.2.4 Comparison with criteria

The available oral LD50 of MBM is within the range of acute oral toxicity category 4 (300-200 mg/kg bw).

No respiratory LC50 is available for MBM, but molar read across from the total releasable amount of formaldehyde (factor 6.2) would result in an vapour LC50 (4h) of 3.7 mg/L corresponding to acute respiratory category 3 (2-10mg/L).

No dermal LD50 is available for MBM, but molar read across from the total releasable amount of formaldehyde (factor 6.2) would result in a dermal LD50 (4h) of 1674 mg/kg bw, corresponding to acute dermal category 4 (1000-2000mg/L).

However classification of corrosive substances for acute toxicity is mechanistically redundant unless non-corrosive concentrations are tested. The latter is also a requirement of the respective OECD test guidelines. Furthermore corrosivity and related LD50 estimates depend on the

concentration of the substance. This may lead to different acute toxicity categories for the same substance dependent on the applied test concentration. Therefore we propose no acute toxicity classification for MBM.

4.2.5 Conclusions on classification and labelling

No classification is required.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

A single oral OECD technical guideline (TG) 423 compliant study in rats was available. No dermal or inhalation studies were presented. In the oral study, the LD_{50} was between 500 and 2000 mg/kg/d. The DS indicated that the respiratory LC_{50} of formaldehyde and the dermal LD_{50} of formaldehyde may be applied to MBM for classification purposes, but in the end it was concluded by the DS that classification of corrosive substances for acute toxicity is redundant.

Comments received during public consultation

Two MSCA suggested that the classifications for acute toxicity are not covered by the classification on skin corrosion and proposed to refer to formaldehyde. Two MSCA suggested to consider classification as acute toxicity 4.
One MSCA suggested additional labelling with EUH071 and EUH 029.

Assessment and comparison with the classification criteria

Acute oral toxicity

Morpholine
The oral LD_{50} in rats varied between 1050 and 1900 mg/kg bw (limited documentation). In guinea pigs an oral LD_{50} of 900 mg/kg bw was observed. There is a minimum harmonised classification of morpholine as Acute Tox. 4* - H 302 (Harmful if swallowed).

Formaldehyde
Formaldehyde has a minimum classification in CLP, Annex VI for Acute oral toxicity, in Category 3* (H301 – Toxic if swallowed).

MBM
Regarding the OECD TG 423 study on MBM, the DS stated that there is evidence from pathology results in rats which died during the post exposure observation period that toxicity of the undiluted test substance after oral administration is due to local effects on the mucous membranes in the gastro-intestinal tract.

The study summary (cf. MBM_Doc IIIA6_1_1) stated that clinical signs at 500 mg/kg bw were seen in males only (hunched posture, lethargy, ataxia, decreased respiratory rate, and laboured respiration) and 1/3 males died (0/3 females). Clinical signs at 2000 mg/kg bw were hunched posture, lethargy, ataxia, ptosis, pilo-erection, prostration, decreased respiratory rate, noisy respiration, tiptoe-gait. At 2000 mg/kg bw 3/3 females died (no males tested). Rats which died during the post exposure observation period revealed 'varying degree of mucosal lesions' in the gastro-intestinal tract (stomach and intestine). No effects were detected in survivors.
No effects were observed in rats receiving the diluted test substance (2%) at 200 mg/kg bw (cf. MBM – Doc III A6.1.1).

From the available acute oral study on MBM it remains unclear whether local effects on the gastro-intestinal tract were the cause of deaths as the general health status was

severely affected and the information of 'varying degree of mucosal lesions' does not allow a conclusion on the cause of death. In addition, classification for corrosivity does not cover the classification for acute toxic effects. Lethalities (from all possible causal effects) in the relevant dose ranges given by the CLP Regulation have to be considered for classification on acute toxicity.

The observed acute toxicity is in line with the observed range of LD_{50} for morpholine which would support classification as Acute Tox. 4.

This conclusion is supported by the findings from a 14-day range-finding study on MBM (cf. Doc IIIA6_3_1). In this study 1 male rat died after dosing on day 2, 1 male at the end
of day 2 and 2 females died on day 3. The application was terminated on day 4 in this dose group. Clinical symptoms at 1000 mg/kg bw/d were similar to those observed in the acute toxicity study (see above). Reddening and haemorrhages of gastric epithelium
and limiting ridge, thinning of the non-glandular gastric epithelium, red intestinal content as well as gaseous distension of the GI-tract was reported at this dose. Hunched posture, noisy respiration and increased salivation was also seen at 250 mg/kg bw/d; no mortalities were seen at this dose and at 50 mg/kg bw/d.

Using data from formaldehyde would result in a corrected LD_{50} of 3840 mg/kg bw (based on the LD_{50} of 640 mg/kg bw for formaldehyde from a non-guideline rat study and following a correction by factor of 6 due to the maximum release of 16.7% formaldehyde from MBM). This alone would not justify classification for acute toxicity of MBM based on data from formaldehyde only. However it should be taken into account that formaldehyde is presently classified as Acute Tox. 3 indicating that lower LD_{50} in mice (LD_{50} 42 mg/kg bw) and guinea pigs (LD_{50} 260 mg/kg bw) from non-guideline studies had been taken into account when the decision on classification was taken.

Data from morpholine may also be considered; morpholine is classified as Acute Tox. 4.

In conclusion, an OECD TG 423 (acute toxic class method) study on MBM revealed an acute toxic estimate (ATE) value of $500 < LD_{50} < 2000$ (m&f combined) which is consistent with the acute mortalities seen at 1000 mg/kg bw/d in a 14-day range-finding study in rats. Thus, RAC agrees to classify as **Acute Tox. 4 - H302 (Harmful if swallowed)** according to CLP (oral ATE values for this category are from > 300 to $\leq$ 2000 mg/kg bw).

Acute inhalation toxicity

Morpholine
Symptoms described in an acute inhalation toxicity study were haemorrhage of the nose, mouth and eyes as well as spasm and tremor in rats; the LC_{50} in male and female rats was reported to be ca. 8 mg/L (but there was limited documentation available to RAC). Similar LC_{50} values for mice are available (6.9 mg/L (f) and 5.2 mg/L (m)). Based on these data the available LC_{50} values would not justify classification, however, there is a minimum harmonised classification for morpholine as Acute Tox. 4* - H 332 (Harmful if inhaled).

Formaldehyde
Formaldehyde is classified in CLP, Annex VI for Acute inhalation toxicity, in Category 3 (H331 – Toxic if inhaled).
There are acute inhalation studies (see Formaldehyde Core Document) suggesting that corrosive effects in the upper respiratory tract may contribute (possibly in addition to other effects) to lethality: Histopathological examination revealed excessive mucus secretion, mucociliary dysfunction, single cell necrosis, and discontinuous nasal epithelium with erythrocyte leakage following 4 h exposure of rats to formaldehyde gas

at concentrations of 12 µg/L (Bhalla et al., 1991). Higher concentrations (0.6-1.7 mg/L) resulted in haemorrhage and oedema of the lung as well as oedema in liver and kidneys and hepatocyte necrosis (Skog, 1950). The Formaldehyde Core Document indicates an LC_{50} of 0.6 mg/L (4 h).

MBM
Studies on acute inhalation toxicity were not available on MBM.
RAC considers using data from formaldehyde justified as MBM contains 16.7% releasable formaldehyde. As proposed by two MSCA during public consultation, classification should be considered based on the data leading to the classification of formaldehyde (in Cat. 3) and taking the maximum amount of releasable formaldehyde into account.
The possible contribution of morpholine (classified as Acute Tox. 4*) to acute inhalation toxicity of MBM is unknown.

Acute Tox. Cat 4 is considered justified based on the assumption that the acute inhalation toxicity of MBM would be totally dependent on 16.7% releasable formaldehyde. For MBM a corrected LC_{50} of about 3.6 mg/L (factor of 6 applied on a LC_{50} of 0.6 mg/L (4h) for formaldehyde) would result. For MBM mists, this is consistent with the criteria in the CLP Regulation for classification as Acute Tox. 4 (LC_{50} guidance values >1 and ≤5 mg/L). Thus, RAC agrees to classify MBM as **Acute Tox. 4 - H332 (Harmful if inhaled)**.

EUH071
The supplemental labelling with the hazard statement EUH071 – Corrosive to the respiratory tract - was proposed by one MSCA. If in addition to classification for inhalation toxicity, data are available that indicate that the mechanism of toxicity is corrosivity (CLP, Note 1 in Table 3.1.3), EUH071 could be assigned.

RAC notes that the CLP criteria for EUH071 are not clearly defined. EUH071 can also be applied to inhaled corrosive substances not tested for acute inhalation toxicity. According to CLP Annex II, Section 1.2.6 (which states *'For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled*') EUH071 may then be appropriate without a corresponding classification for acute inhalation toxicity.

In line with previous RAC recommendations (including those on other formaldehyde releasers) where EUH071 has been assigned in addition to the classification for acute inhalation toxicity and based on the corrosive properties of both hydrolysis products (formaldehyde and morpholine) which, in addition to other possible mechanisms, may have contributed to mortalities, **RAC agrees to assign EUH071 toMBM**.

EUH029
The labelling EUH029 - Contact with water liberates toxic gas - was suggested for consideration by one MSCA. CLP, Annex II, Section 1.2.1 defines substances and mixtures which in contact with water or damp air, evolve gases classified for acute toxicity in category 1, 2 or 3 in potentially dangerous amounts.

RAC emphasises that the liberation of toxic gas after contact with water will not be a major concern as sufficiently high amounts of toxic gas may not be produced immediately. Formaldehyde will also be generated and released without direct contact with water as aqueous conditions arise under normal room air conditions following contact with mucous membranes (of the eye, the respiratory tract and the upper gastrointestinal tract) and in contact with sweaty skin. **RAC agrees that EUH029 is not warranted**.

It is also noted that the CLP Regulation (Annex II, Section 1.2.1) provides for the additional labelling with EUH029 only for substances classified for acute toxicity in

category 1,2 or 3 and not for Acute Tox. Cat.4 substances.

Acute dermal toxicity

Morpholine
Acute dermal toxicity studies in the rabbit revealed an LD_{50} of 500 mg/kg bw (non-guideline study, 1954). In Annex VI of the CLP Regulation, there is a harmonised minimum classification for acute toxicity for morpholine as Acute Tox. 4* - H312 (Harmful in contact with skin).

The data could be considered to support classification of morpholine as Acute Tox. 3 – H 311 (Toxic in contact with skin). The applicant presumed that death was caused by the corrosive effects of morpholine (cf. MBM_Doc III App. Morpholine). This conclusion was based on the observation that all of the 7 animals that received a repeated dermal dose of 900 mg/kg morpholine (at 33% in an aqueous solution) died before the 11^{th} dose. In this study the skin was reported to be necrotic, having a thickened oedematous area under the application site; the underlying organs showed inflammation and congestion (Shea, 1939, no further data documented in the MBM_Doc III App. Morpholine).
However, from the acute dermal data (with lack of data indicating local skin effects) and from the repeated dermal data (with lack of data on systemic effects that could have contributed to the deaths) no clear conclusion can be drawn on which effects caused the acute mortalities resulting in the LD_{50} dose of 500 mg/kg.
The corresponding concentration in the acute test on rabbits that received 500 mg/kg bw (4 h) was calculated to be 18.3% (based on the information from the repeated dose study) which is much lower than the testing of pure substances in testing on skin irritation/corrosion and make it more unlikely that corrosive effects were the only cause of the observed deaths in the study on rabbits.

Formaldehyde
Formaldehyde is classified in CLP, Annex VI for Acute dermal toxicity, in Category 3 (H311 – Toxic in contact with skin).

MBM
Studies on acute dermal toxicity were not available on MBM.
RAC considers that for MBM data for morpholine and formaldehyde can be used. Based on the dermal LD_{50} (4 h) for formaldehyde (270 mg/kg bw), which, corrected with a factor of 6 the corresponding LD_{50} for MBM is 1620 mg/kg bw, which is in the range > 1000 mg/kg and <2000 mg/kg for Category 4.

This category corresponds to the current harmonised (minimum) classification of morpholine as Acute Tox. 4*. Based on the dermal LD_{50} of 500 mg/kg bw for morpholine and taking no correction factor for the maximum releasable amount of morpholine into account (since in the presence of water 100 mg MBM will produce 93 mg morpholine), this value would, however, correspond to Acute Tox. 3 (guidance value for Category 3 > 200 and ≤1000 mg/kg). It may be noted that the available dermal studies on formaldehyde and on morpholine were conducted before 1981 and have limitations in comparison with currently available technical guidelines.

RAC agrees that based on the data from formaldehyde and the corrected acute dermal LD_{50} value of 1620 mg/kg bw (based on formaldehyde), and taking into account the harmonised classification of morpholine as Acute Tox. 4 and some uncertainties (based on the available summary information from a study published in 1954) from the acute dermal toxicity study on morpholine, MBM should be classified as **Acute Tox. 4 - H312 (Harmful in contact with skin)** according to CLP (dermal LD_{50} criteria in the CLP Regulation for this category are from 1000 to 2000 mg/kg bw).

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

MBM should be classified for corrosion, additional labeling for STOT SE 3 (respiratory irritation) would be redundant. Besides corrosive or irritant effects at the site of contact no other specific target organ toxicities are observed or expected.

Therefore no classification is required.

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

Summary of the Dossier submitter's proposal

The DS has argued that there is no evidence for effects justifying STOT SE 1 or 2 and that STOT SE 3 - H335 is not appropriate as the substance is corrosive.

Comments received during public consultation

There was no comment that supported classification for STOT SE. One MSCA agreed that no classification for STOT SE3 is required and commented that respiratory irritation is covered by the classification as acutely toxic and/or corrosive.

Assessment and comparison with the classification criteria

Morpholine
For morpholine, there is no entry in Annex VI to the CLP Regulation for STOT SE.

Formaldehyde
For formaldehyde, there is no entry in Annex VI to the CLP Regulation for STOT SE; some notifiers have self-classified for STOT SE.

MBM
Based on the acute toxicity data on MBM there were no effects beyond those covered by the classifications on acute dermal and oral toxicity that would justify STOT SE 1 or 2.

There are no experimental/other data that justify an additional classification as STOT SE 3 (H335) for respiratory tract irritation, and the CLP Guidance 3.8.2.5, states as follows

> *'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. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed.'*

Based on the CLP criteria, STOT SE 3 should also be considered as covered by the classification as skin corrosive.

RAC agrees with the DS that **no classification on STOT SE is warranted**, and that the potential for respiratory tract irritation is covered by the classification of MBM as corrosive to the skin.

4.4 Irritation

4.4.1 Skin irritation

No specific guideline studies are available for Octanoic or Decanoic acid. However sufficient publications are available to assess the irritation potential by a total weight of evidence approach.

4.4.1.1 Human information for MBM

Not available.

4.4.1.2 Non-human information for MBM

Table 4.4-1 Skin irritation data for MBM

Species	Method	Average score 1, 24, 48, 72 h after patch removal		Reversibility	Result / remarks	Reference
		Erythema	Edema	yes/no		
Rabbit	OECD 404; undiluted test substance; exposure time = 4 hours; semi-occlusive condition; 3 rabbits	1h: score 3 in all 3 rabbits 24 h: score 4 in 1 rabbit; score 3 in 2 rabbits. Thereafter evaluation of erythema was prevented by: hardened dark brown/black-coloured scab, scab undulating, or sunken hardened brown/black-coloured scab resembling a crater. Day 9 the animals were killed for humane reasons due to reactions indicative of dermal corrosion.	1h: score 4 in all 3 rabbits 24 h: reaction extends ca. 4 cm beyond the treatment site and ventrally below the treatment site. Thereafter evaluation of oedema was prevented (see erythema).	no	clear evidence for irreversible tissue damage Nowadays available and validated in vitro skin corrosion or irritation tests should be used. However the study was carried out in 2001, before the OECD acceptance of the in vitro skin corrosion tests.	Lubrizol (2001), MBM-Doc III A6.1.4

4.4.1.3 Products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.3 & Formaldehyde – Doc III A6.1.4)

The database for evaluation of skin irritation of aqueous solutions is limited. In rats there is "erosion" of the skin at 7-9% aqueous solutions. Consequently formaldehyde is classified with R34 (causes burns, Dir. 67/548/EEC) or H314 (Causes severe skin burns and eye damage, Reg. No 1272/2008).

Morpholine (Morph-Doc II A4.3 & Morph-Doc III A 6.1.4. Additional Information)

Literature evidence supports classification of undiluted morpholine with R34 (causes burns, Dir. 67/548/EEC) or H314 (Causes severe skin burns and eye damage, Reg. No 1272/2008). No effects were detected in guinea pigs when the dose was neutralized with sulphuric acid suggesting that effects are related to the high pH value.

A 2% solution in water caused skin irritation after 72 h but not after 0.5, 24 or 48 hours in rabbits. 10% morpholine in white petrolatum has no skin irritant effects in guinea pigs when applied for 24 hours (Wang & Suskind, 1988).

From these data a short term NOAEC of 2% for local effects on rabbit skin may be proposed.

4.4.1.4 Comparison of MBM with products of hydrolysis

Table 3.3-2. Comparison of data on skin irritation

MBM	Formaldehyde	Morpholine
Causes burns	Causes burns	Causes burns

4.4.1.5 Summary and discussion of skin irritation

The irritant/corrosive effects on the skin were studied in rabbits. The undiluted active substance caused severe irreversible tissue damage (cf. MBM – Doc III A6.1.4). These data support classification and labelling of MBM with H314 (Causes severe skin burns and eye damage).

In preliminary experiments of the sensitization study (cf. MBM-Doc III A6.1.5) necrosis was found in 4 of 6 guinea pigs when MBM was applied in the vehicle Alembicol D (product of coconut oil) for 24 hours occlusive at a concentration of 20%, irritant effects were detected in 5 from 6 animals at a concentration of 10%, in one from 6 animals grade 1 erythema was observed at 5% and at 1%. However, in the main test no erythema or edema appeared at concentrations ≤ 1% in any of the 30 animals (3/30 animals with 5%). In acute oral toxicity studies no irritant effects were observed by macroscopic necropsy in rats receiving via gavage the diluted test substance at a concentration of 2% in water (10 ml/kg bw or 200 mg/kg bw; cf. MBM – Doc III A6.1.1). From these data an acute NOAEC of 1% for local effects on rat skin may be supported.

4.4.1.6 Comparison with criteria

The undiluted active substance caused severe irreversible tissue damage as summarized in table 4.4-1 above (cf. MBM – Doc III A6.1.4). The data demonstrate corrosive potential but do not allow differentiating between sub-categories, since only a 4 hour exposure was applied. Nevertheless, based on the old system the substance causes burns and warrants the R-phrase 34. In the old categorisation system category 1C is not foreseen, thus, it is recommended to sub-categorise the substance with category 1B according to the GHS criteria.

4.4.1.7 Conclusions on classification and labelling

The data support classification and labelling of MBM in Skin Corrosive Category 1B, H314 -Causes severe skin burns and eye damage.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One OECD TG 404 in rabbits was presented in the CLH report. The study indicated that the test substance is corrosive. The data make subcategorisation difficult but as the option with category 1 without subcategorisation is not currently in the legal text the DS proposed classification as Skin Corr. 1B.

The DS noted that the hydrolysis products of the substance, formaldehyde and morpholine, are also corrosive.

Comments received during public consultation

Two MSCA disagreed with the subcategorisation, however, they apparently were not aware that the the CLP Regulation required subcategorisation.

Assessment and comparison with the classification criteria

Morpholine
Morpholine is classified in Annex VI to the CLP Regulation, as Skin Corr. 1 B, H314 'Causes severe skin burns and eye damage'.

Formaldehyde
Formaldehyde is classified in Annex VI to the CLP Regulation, as Skin Corr. 1 B, H314 'Causes severe skin burns and eye damage'.

MBM
Corrosive effects of undiluted MBM were seen in all of 3 rabbits in an OECD TG 404 study at 24 h after a 4 h exposure. No information is available on effects at shorter exposure times as testing was conducted before 2002 i.e. before the relevant OECD TG was published.

Although testing data with exposure for 3 min and 1 h were not available, and it could not be demonstrated whether skin necrosis would have developed after shorter exposure time than after 4 h, RAC propose that data from the hydrolysis products formaldehyde and morpholine should be applied for MBM.
The observation that necrosis was noted after 24 h after the end of exposure to MBM does not exclude the possibility that necrosis could also occur during the post-exposure observation period after exposure for ≤ 1 h.

RAC took note of the difficulties identified by the DS and MSCA concerning on the selection of the appropriate a subcategory. As subcategorisation is required based on the

CLP Regulation, RAC agrees to refer to the data for formaldehyde and morpholine which both are classified as Skin Corr. 1B – H 314 'Causes severe skin burns and eye damage'. The same classification is warranted for MBM**. RAC thus agrees that Skin Corr. 1B – H314 (Causes severe skin burns and eye damage) is warranted.**

4.4.2 Eye irritation

No data specific for eye irritation are available for MBM. However MBM should be classified for skin corrosion Cat 1B. No further classification for local eye effects is necessary.

4.5 Corrosion

See chapter 4.4

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

No data were available, but the DS concluded that classification as skin corrosive also covers eye effects.

Comments received during public consultation

One MSCA proposed classification for serious eye damage (Eye Dam.1) but no labelling as explained in CLP Guidance, Chapter 3.3.2.4.

Assessment and comparison with the classification criteria

Morpholine

For morpholine the labelling 'H314 – Causes severe skin burns and eye damage' covers the potential for eye damage. There is no separate classification for eye damage in Annex VI of the CLP Regulation, but many notifiers have self-classified the substance as Eye Dam. 1.

Formaldehyde

There is no Annex VI entry on a separate classification for eye irritation/damage on formaldehyde, however the majority of notifiers have self-classified the substance as Eye Dam. 1.

The Formaldehyde Core Dossier summarises that although no guideline-conforming testing has been conducted, testing on dilutions (up to 15%) indicated severe irreversible eye damage that would justify the classification as Eye Dam. 1.

Due to specific concentration limits assigned to the existing Annex VI entry, mixtures containing formaldehyde at concentrations within the range 5% ≤ C < 25% are classified as Eye Irrit. 2; H319.

In humans, indications of eye irritation such as increased eye blink frequency and conjunctival redness were seen from gaseous concentrations of 600 µg/m³ (WHO 2010).

MBM

With regard to the comment from one MSCA, CLP Guidance is not clear with regards to a separate classification for corrosive effects on the eye.

CLP Guidance stipulates in Section 3.3.2.4:

A skin corrosive substance is considered to also cause serious eye damage which

is indicated in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required but the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27).

However, the first sentence of CLP Guidance, Section 3.3 recommends:

It should be noted that if a substance or mixture is classified as Skin corrosive Category 1 then serious damage to eyes is implicit and there is no need to proceed with classification for eye effects.

In line with previous decisions where a separate classification on eye damage may be considered if separate studies on eye effects are available and had demonstrated irreversible eye damage, **no classification for irreversible eye effects is warranted for MBM**. Studies on eye irritation are not available for MBM. The classification of MBM as Skin Corr. 1B coupled with the labelling with H314 (Causes severe skin burns and eye damage) covers corrosive effects on the eyes.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information for MBM

Table 4.6-1: Summary of animal skin sensitisation data for MBM

Species	Method	Number of animals sensitized/total number of animals	Result / Remarks	Reference
Guinea pig	Guinea pig maximization test according to OECD 406	3/20; were not considered to represent hyper-sensitivity	Inconclusive (concentration for induction too low)	Lubrizol (2001) MBM-Doc III A6.1.5

4.6.1.2 Human information for MBM

Not available.

4.6.1.3 Products of hydrolysis

Formaldehyde (Formaldehyde - Doc II A3.4 & Formaldehyde - Doc III A6.1.5)

Formaldehyde is a known primary skin sensitiser inducing Type IV allergic contact dermatitis. The sensitising properties of formaldehyde are confirmed by a large number of tests in laboratory animals, including the guinea pig maximisation test according to Magnusson & Kligman. The specific design of the LLNA reported by Basketter et al. (2001) allowed estimation of the concentration required for tripling of lymph node lymphocyte proliferation – a measure regarded as threshold effect – as approx. 0.35 % formaldehyde in an acetone/olive oil vehicle. Other considerations suggested a threshold concentration for induction below 5 % in solution and a low probability for positive reactions in humans below 0.025-0.05 %. However, the currently available methodology as well as the submitted database is not suitable for derivation of a NOAEC for sensitisation by formaldehyde which is relevant to human health. Nevertheless, the available data is in support of the current ECB classification and labelling limit for formaldehyde formulations of ≥ 0.2 % (w/w) with regard to its sensitising properties.

Specialized studies on experimental animals did not show sufficient evidence for respiratory sensitization. Human data on respiratory sensitization gave no clear evidence for formaldehyde-induced asthma.

Morpholine (Morph-Doc II A4.4 & Morph-Doc III A6.1.5 Additional Information)

No valid data on sensitizing effects in experimental animals are available. In a Buehler assay no sensitizing effects were found in 10 male Hartley guinea pigs; but the experimental design of this test was invalid. The available data in humans are insufficient for evaluation.

4.6.1.4 Comparison of MBM with products of hydrolysis

Table 3.4-2 Comparison of data on sensitization

Endpoint	MBM	Formaldehyde	Morpholine
Sensitization in experimental animals	Data inconclusive	Sensitizing	Data not sufficient for assessment
Sensitization in human	No data	Sensitizing	Data not sufficient for assessment

4.6.1.5 Summary and discussion of skin sensitisation

In a guinea pig maximization test according to current guidelines no sensitizing effects of MBM were observed. (Lubrizol, 2001; cf. MBM-Doc III A6.1.5). There have been problems in the study in achieving the right concentration of MBM during induction: During intradermal induction only in 4/20 animals the irritation was higher than in controls. The topical induction (10 %) did not cause irritation in any of the animals. Since it cannot be excluded, that doses leading clearly to irritation during induction might have induced sensitization this study is considered as inconclusive.

However formaldehyde is a well-known human skin sensitizer. MBM may be classified based on mechanistic considerations of total releasable amount of formaldehyde upon contact with biological media and read across of the sensitizing property of formaldehyde. Sub-categorisation into category 1A or 1B is not possible, since also formaldehyde is not sub-categorised.

However the specific classification limit of formaldehyde (0.2%) was read across on a molar basis (factor 6.2) and proposed as 1.2%.

Alternatively MBM may not be classified for skin sensitization based on considering just the amount of free formaldehyde in MBM.

4.6.1.6 Comparison with criteria

Arguments to support classification of MBM on the basis of total releasable formaldehyde and alternatively on free formaldehyde content are presented in chapter 4.10 on carcinogenicity.

4.6.1.7 Conclusions on classification and labelling

Classification for dermal sensitization cat 1 is proposed. A specific classification limit of 1.2% is proposed.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal There was one inconclusive OECD TG 406 (GPMT) study summarised in the CLH report and no human data were available. The DS proposed either classification based on the calculated release of formaldehyde by the substance or no classification based on the amount of free formaldehyde. An SCL was calculated based on the SCL for formaldehyde and the fraction of the substance that could be released as formaldehyde. **Comments received during public consultation** Four MSCA supported the classification. However, the proposed SCL was questioned and

the DS agreed in the response to MSCA comments that the SCL should be removed. Industry disputed the classification proposal claiming that there is no data on the substance supporting classification.

Assessment and comparison with the classification criteria

Morpholine
No sensitising effect was observed in a Buehler assay with invalid test design.

Formaldehyde
The existing classification of the hydrolysis product formaldehyde in Annex VI is Skin Sens. 1; H317 with a specific concentration limit of ≥0.2%.

MBM
A GPMT test (OECD TG 406) on MBM revealed 3/20 responders after challenge with 5%. MBM. A positive rate below 30% would normally not justify classification, however the test was judged as inconclusive as only one very low concentration (0.1%) was tested for intradermal induction. The study authors did not consider the effects as indication of hypersensitivity as the topical induction concentrations up to 10% MBM did not cause irritation. No indication of slight irritation (no erythema or oedema) appeared at topical (challenge) concentrations of 1% MBM in any of the 30 animals in the main study. This was in contrast to the observation that 1/6 animals at 1% and 5% MBM had grade 1 erythema in the preliminary studies. Irritant effects were seen in 5/6 animals at 10% in the pilot study, but this concentration was not tested in the main study as a challenge concentration.

The DS highlighted that the dermal absorption rate of MBM was estimated at 60-70%, which is similar to that of formaldehyde (40-65%).
RAC is of the view that there ispotential for formaldehyde to be produced at the skin surface after hydrolysis in contact with (sweaty) skin and this will then be absorbed, or formaldehyde may be produced following dermal absorption of MBM. Read across to formaldehyde (without subcategorisation) and classification of MBM as **Skin Sens. 1; H317 (May cause an allergic skin reaction) is proposed by RAC**.

The initial proposal of the DS to correct for the amount of releasable formaldehyde is also considered. A correction of the SCL of formaldehyde with a factor of 6 would result in a SCL of 1.2% for MBM. This is in the range of the general concentration limit and instead of calculating a theoretical SCL **RAC proposes** (in agreement with the DS and MSCA, see RCOM comment No. 19) **to apply the generic concentration limit for a Category 1 sensitiser**.

4.6.2 Respiratory sensitisation

No data are available.

4.7 Repeated dose toxicity

4.7.1 Non-human information MBM

Table 4.7-1 Repeated dose toxicity of MBM

Route	duration of study; guide-line	Species Strain Sex no/group	dose levels frequency of application	Results / Remarks	LO(A)EL	NO(A)EL	Reference
Oral (gav-age)	14 Days; no	Rat Sprague-Dawley 5 m & 5 f	0, 50, 250, 1000 mg/kg bw; once daily 7 days per week (concentra-tion 0, 2.5, 12.5, 50%)	Dose range finding study Local effects in the stomach (thickening of the non-glandular part) observed even at the lowest dose; high dose: increased mortality, do-sing terminated on day 4	50 mg/kg bw	< 50 mg/kg bw	Lubrizol (2002a) MBM-Doc III A6.3.1
Oral (gav-age)	90 Days; OECD 408	Rat Sprague-Dawley 10 m & 10 f	0, 5, 15, 50, 250/150 mg/kg bw; once daily 7 days per week; con-centration: 0, 0.25, 0.75, 2.5, 12.5 / 7.5% in oil	Local effects in the forestomach & glandular stomach at ≥ 50 mg/kg bw	50 mg/kg bw (corres-ponds to 2.5% in oil)	15 mg/kg bw (corres-ponds to 0.75% in oil)	Lubrizol (2002b) MBM-Doc III A6.4.1

4.7.2 Human information - MBM

Not available.

4.7.3 Products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.5 & Formaldehyde – Doc III A6.3-6.5)

There is evidence that formaldehyde induces toxic effects at the site of contact after oral, dermal or inhalation exposure. Repeated dose studies with Formaldehyde are largely poorly documented with regard to effects other than those at the port of entry. However it is accepted that general signs of toxicity are very likely to occur only secondarily to these local lesions and at high formaldehyde concentrations.

The main effects in chronic drinking water studies with rats are local lesions in the forestomach and the glandular stomach. A short, medium and long term NOAEL of 15 mg/kg bw/day (NOAEC 0.026%) is derived as conclusion from several studies.

Data available on repeated dermal exposure are of limited validity due to restricted documentation. A short and medium term dermal NOAEC of 0.1% is reported as conclusion of several studies. This NOAEC may be considered to be in line with the actual skin sensitization classification limit of 0.2% for formaldehyde. However the data basis for the derivation of a robust local dermal short, medium and long term AEL is not considered to be sufficient.

A large number of repeated inhalation studies and also human data are available. A local inhalatory acceptable short, medium and long term exposure concentration was derived for Formaldehyde as 0.12 µg/L based on a sufficient data set including concern for carcinogenicity and –as most sensitive endpoint- human eye irritancy.

Morpholine (Morph-Doc III A6.3-5 Additional Information & Morph-Doc III A6.4.3 & Morph-Doc III A6.5.3)

Repeated oral exposure to MOAS (morpholine oleic acid salt; 0.15, 0.3, 0.6, 1.25, and 2.5% MOAS

MOAS in drinking water daily ad libitum) in sub-chronic studies in mice resulted in renal effects with a NOAEC of 0.3% MOAS corresponding to 154 mg/kg bw day morpholine. In a chronic drinking water study with mice only a body weight reduction of females was observed with 0.06% or 128 mg/kg bw day morpholine, however the influence of reduced water consumption is unclear. Morpholine concentrations of 0.25% or doses of 385 mg/kg bw day induced in addition local forestomach effects and blood urea nitrogen increase (protocols comparable to OECD sub-chronic and chronic study protocols).

Data on repeated dermal exposure are insufficient for final evaluation but suggested strong local effects at the site of 1st contact. With neutralisation by sulphuric acid local effects were minimal also after 30 days of daily repeated exposure.

In a sub-chronic as well as in a chronic inhalation study in rats no systemic effects were found but irritation of eyes and nasal cavity. In the chronic study infiltrates, metaplasia and necrosis of the nasal cavities were detected at ≥ 181 mg/m³ (NOAEC 36 mg/m³).

4.7.4 Comparison of MBM with products of hydrolysis

Table 4.7-2 Comparison of data on repeated dose toxicity

Parameters	**MBM**	**Formaldehyde**	**Morpholine**
Dermal exposure Study duration Species LOAEL (mg/kg bw/day) NOAEL (mg/kg bw/day)	No data, but local effects expected	Local effects, data not sufficient for assessment	Local effects, data not sufficient for assessment
Inhalation exposure effects Study duration Species LOAEC (mg/m³) NOAEC (mg/m³)	No data, but local effects expected	Local effects - eye irritancy long term (lit. review) human 0.12	Local effects 104 weeks rat 181 36
Oral exposure Study duration effects Species LOAEL (mg/kg bw/day) NOAEL (mg/kg bw/day)	Via gavage 90 days local effects rat 50 (LOAEC 2.5%) 15 (NOAEC 0.75%)	Via drinking water 2 years local effects rat 82 (m) or 109 (f) (0.19%) 15 (m) or 21 (f) (0.026%)	Via drinking water – MOAS, but values given for morpholine 96 weeks / 90 days local fore-stomach / renal effects mouse 385 (LOAEC 0.25%) / 205 128 (LOAEC 0.06%) / 154

MOAS: morpholine oleic acid salt

4.7.5 Summary and Discussion of repeated dose toxicity

In a subacute oral dose-range-finding study in rats local toxic effects of MBM in the stomach were noted at 50 mg/kg bw/day or a concentration of 2.5% in arachis oil (volume 2 ml; cf. MBM-Doc III A6.3.1).

In the subchronic gavage study in rats receiving 0, 5, 15, 50, 250/150 mg/kg bw/day or 0, 0.25, 0.75, 2.5, 12.5/7.5% in a total volume of 2 ml arachis oil (cf. MBM-Doc III A6.4.1) only local effects were found. These effects have been detected mainly in the fore-stomach but also in the glandular stomach. Acanthosis, hyperkeratosis and inflammation in the forestomach occurred in males and females at ≥ 50 mg/kg bw/day, males seem to be less susceptible at 50 mg/kg bw than females. A few males and females of the high dose group revealed also effects in the oesophagus. This inflammation reaction is not discussed by the authors as treatment related but there is some indication for such local effects.

In the upper respiratory tract treatment related effects (mainly inflammatory changes) were observed. In males and females similar effects were found in the nasopharynx and in females additionally in larynx and

trachea. In females effects in the upper respiratory tract occurred only at the high dose level but in males effects were seen at ≥ 15 mg/kg bw/day (inflammation in the nasopharynx of 2/10 males).The authors of the study suggested that these effects in the upper respiratory tract are not systemic effects but a consequence of accidental instillation or otherwise direct action of the test substance during application. The same conclusion was given in an additional evaluation (cf. MBM-Doc III A6.4.1). It might be due to contamination with the corrosive test material in the region of the epiglottis during entry or exit from dosing, which would spread to higher (nasopharynx) and lower (larynx) areas. This view was supported by the fact that the effect was not dose related and that it was most pronounced in the animals with premature deaths.

Reversibility of the local effects in stomach and upper respiratory tract has been demonstrated in recovery groups. However, the effects in the stomach were still present but were of less severity.

Local effects are considered to be concentration dependent. The LOAEL was 15 mg/kg bw in males and 50 mg/kg bw in females, the NOAEL was 5 and 15 mg/kg bw, respectively. If one does not take into consideration the effects in the nasopharynx, which are assumed to be due to unintentionional deposition of the corrosive test substance, the overall LOAEL is 50 mg/kg bw and the NOAEL is 15 mg/kg bw corresponding to 2.5% and 0.75% respectively. However no systemic effects occurred at any dose levels up to the highest dose of 250 mg/kg bw.

In a gavage study in rabbits the teratogenic properties were examined (cf. MBM-Doc III A6.8.1). Rabbits were gavaged with 0, 10, 30, 100 mg/kg bw/day at gestation day 6-28. The doses correspond to 0, 1, 3, or 10% test substance in corn oil. The application volume was 1 ml/kg bw. The test substance induced local effects in the stomach of dams. Significant increase in stomach lesions (e.g. erosion and granula aspect of stomach) at necropsy were detected at ≥ 30 mg/kg bw/day. In this study the LOAEC for local effects in the stomach is 3% and the NOAEC 1% which is very similar to the effective concentrations in the rat study.

The implementation of a subchronic oral study in a 2nd species is scientifically unjustified because local concentration dependent effects are expected which have been sufficiently demonstrated. Furthermore, the implementation of a sub-acute & sub-chronic dermal toxicity study in rats is scientifically unjustified because of the corrosive properties of MBM and the sensitizing properties of the released formaldehyde. For the latter property no induction NOAEC can be derived yet.

4.7.6 Comparison with criteria for STOT RE

For MBM data on repeated dermal application are lacking. However, due to the corrosive properties of MBM a repeated dose toxicity study with dermal application is not justified. Chronic studies are available for formaldehyde and morpholine and these studies indicated local effects at the site of contact for both substances.

No repeated dose inhalation studies with MBM are available. However based on the hydrolysis study and the toxicokinetic study it is plausible that the equilibrium of MBM and formaldehyde quickly shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media. Therefore the human data based local inhalative AEC of 0.12 mg/m³ for formaldehyde may be read across to MBM (on molar basis, factor 6.2) and used for assessing the risk from inhalation exposure (see Doc IIA3.12.1).

With repeated oral gavage dosing in rats and rabbits MBM as well as the hydrolysis products formaldehyde and morpholine induced local effects at the site of contact, i.e. in the stomach. In both studies very similar effective concentrations were found: LOAEC 2.5% and 3%, respectively. The corresponding LOAELs were 50 mg/kg bw day and 30 mg/kg bw day. These LOAELs are within the guidance value range for STOT-RE 2 (oral, 10-100 mg/kg bw day). The LOAELs are also “more than half an order of magnitude lower that mediating the evident acute toxicity”, the oral LD50 (see chapter 3.9.2.5.1 in ECHA CLP guidance 2012).

However it is considered that the observed local, irritating effects should not support the classification for STOT RE, since the available mechanistic information on hydrolysis to formaldehyde and local denaturation of organic tissue supports that the local effects are mechanistically already sufficiently addressed with the classification for corrosion/irritation.

4.7.7 Conclusions on classification and labelling for STOT RE

No classification necessary for STOT RE is required.

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

Summary of the Dossier submitter's proposal

One OECD TG 408 and one 14-day non-guideline study were presented. In both studies there where effects in the fore-stomach and in the glandular stomach. The effects were seen at doses that could warrant classification as STOT RE 2, but the DS concluded that the effects were due to the corrosive action of the substance and thus suggested no classification.

Comments received during public consultation

Two MSCA suggested that classification for STOT RE is warranted.

Assessment and comparison with the classification criteria

Oral route

Morpholine

Repeated oral exposure to MOAS (morpholine oleic acid salt; 0.15%, 0.3%, 0.6%, 1.25% and 2.5% MOAS in drinking water daily *ad libitum*) in sub-chronic studies in mice (OECD TG 409) corresponded in males to 77, 154, 256, 436 and 795 mg/kg bw/d morpholine, respectively, and in females to 77, 128, 205, 410 and 667 mg/kg bw/d morpholine, respectively. Treatment resulted in lower body weight gain (at the highest dose), increased blood urea nitrogen and increased specific gravity of urine (≥0.6% MOAS), increased renal weight (≥1.25% MOAS), and cloudy swelling of proximal renal tubules (at the highest dose). The study authors concluded that MOAS produced a mild toxic nephrosis, while the applicant considered the kidney effects to have been related to decreased water consumption. A decrease in water consumption (that was not reported in the study summary) may explain the higher specific gravidity, but is unlikely to be responsible for the increased blood urea nitrogen. This effect indicates damage to the renal tubuli.

Although there is evidence on kidney toxicity by MOAS, the effect levels were clearly above the guidance level for classification as STOT RE.

In a chronic drinking water study on MOAS with mice (96-weeks followed by a post-observation period of 8 weeks), a lower body weight gain was observed in females at ≥ 0.06% (128 mg/kg bw/d morpholine) and in males at 0.25% (385 mg/kg bw/d), however the influence of reduced water consumption is unclear. Morpholine concentrations of 0.25% (385 mg/kg bw/d) induced local forestomach effects and blood urea nitrogen increase in male mice.
Some evidence on kidney toxicity was confirmed, however the dose range is not relevant for classification. The examination at week 8 after the end of treatment limits the reliability of the study.

Formaldehyde

There is no harmonised classification on formaldehyde for STOT RE.
Lesions related to the irritancy in the stomach are - similar to MBM - the main effects after repeated oral administration of formaldehyde. However, available studies suggest that the lesions were seen at comparatively higher doses or occurred with lower severity grades.

After 12 months exposure to 300 mg/kg bw/d, forestomach squamous cell hyperplasia/hyperkeratosis, glandular hyperplasia and erosion/ulceration of the glandular stomach were seen (Tobe et al. 1989, formaldehyde core Doc III A6.3.1). No local effects

in the gastrointestinal tract were observed in a 90-day study in rats receiving drinking water with formaldehyde up to concentrations of 1000 mg/L (150 mg/kg bw/d) (Johannsen et al., 1986). A 4-week oral study on rats (Til et al., 1988, formaldehyde core Doc III.A6.3.1) receiving 0, 5, 25 or 125 mg/kg bw/d with drinking water revealed at 125 mg/kg bw/d very slight to moderate hyperkeratosis of the forestomach (all animals) and very slight to moderate gastritis (3/10 males, 5/10 females) of the glandular stomach. A focal papillomatous hyperplasia was observed in one female. None of the available studies conducted were fully compliant with the relevant guidelines.

MBM

A 14-day range-finding test on MBM (cf. Doc IIIA6_3_1) revealed acute mortalities at 1000 mg/kg bw/d until day 3 of treatment that were considered for the assessment of acute toxicity. At 250 mg/kg bw/d no mortalities occurred and clinical findings were hunched posture, noisy respiration and increased salivation. Other findings (also at the lower dose of 50 mg/kg bw/d) were increased neutrophils, thickening of the non-glandular stomach, pale kidneys and increased kidney weights in males and females. There were no data on histopathology (note:the kidney was also a target organ in subchronic studies on morpholine in mice).
After correction for the study duration the adverse effects at 50 mg/kg and 250 mg/kg (corresponding to 25 and 125 mg/kg bw/d for a 28-day test design) are supportive for classification as STOT RE 2.

In a subchronic gavage study consistent with OECD TG 408 in rats receiving 0, 5, 15, 50 or 250/150 mg/kg bw/d (cf. MBM-Doc III A6.4.1) hunched posture and noisy respiration were observed in some rats at 50 mg/kg; 1 male died at day 29. Physical condition was severely deteriorated in rats at 250 mg/kg, but reducing the dose to 150 mg/kg bw/d did not result in noticeable improvements. Six females and 4 males of this dose group died between day 13 and day 90. A decrease in body weight was noted in decedents prior to death. A significantly increased urine volume was noted in this group, while no effect was seen on water consumption.
From 50 mg/kg bw/d onwards lesions were detected mainly in the fore-stomach but also in the glandular stomach. Acanthosis, hyperkeratosis and inflammation in the forestomach occurred in males and females at ≥ 50 mg/kg bw/d; males seem to be less susceptible at 50 mg/kg bw than females. At 250/150 mg/kg bw/d ulceration of the stomach was observed in 6/6 surviving male rats and in 3/4 decedent males and 1/6 decedent female. A few males and females of the high dose group revealed also effects in the larynx (hyperkeratosis, hyperplasia and inflammation) and oesophagus (inflammation). This inflammation reaction was not considered by the authors to be treatment related but there is some indication for such local effects. Minimal lymphoid atrophy of the thymus, mesenteric lymph nodes and spleen were observed in some females and males among the unscheduled deaths.
With a tendency for higher incidences with dose, inflammation, necrosis, fibrosis, ulceration and epithelial hyperplasia of the nasopharynx was observed in a number of animals from 15 mg/kg bw/d and above. Although such effects should normally not occur in a gavage study it was explained as being related to accidental application during dosing or related to the gavage dosing.
The observed delayed mortalities and lesions on the gastrointestinal tract (including larynx and oesophagus) at 50 mg/kg bw/d and above are considered to warrant classification (although this is a borderline case). The effects were most prominent at 250/150 mg/kg bw/d, which is above the guidance value of 100 mg/kg bw/d (see Table 3.9.2-a of the CLP Guidance), but started at 50 mg/kg bw/d (including one delayed mortality). No data are available on the dose range > 50 mg/kg and ≤ 100 mg/kg bw/d.

The biocide applicant considered the observed effects as related to exposure to the hydrolysis products formaldehyde and morpholine.

The CLP Guidance does not suggest that effects on the tissues along the administration routes resulting from repeated exposures are covered by classification for corrosion, while it gives some recommendation concerning Annex I 3.9.1.6, when STOT SE might be more appropriate than STOT RE:

> *Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.*

In addition, CLP Guidance, Section 3.9.2.5.1 gives guidance on the doses, as follows:

> *If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity.*

The dose at which the effects in the gastrointestinal tract occurred in the 90-day study was lower than the oral acute toxic doses (1/3 males and 0/3 females died at 500 mg/kg, 3/3 females died at 2000 mg/kg). Local effects in the stomach of varying degree were observed in the oral acute toxicity study (test substance was undiluted, no further information available on any dose-response relationship of the lesions). RAC, in line with comments during the public consultation from two MSCA does not agree with the view of the DS that the local irritant effects are mechanistically sufficiently addressed with the classification for corrosion and should not support the classification for STOT RE.

The delayed mortalities (day 13 – 90) and the toxic effects in the gastrointestinal tract are considered as chronic toxic effects that resulted from prolonged/repeated exposure to low concentrations/doses of MBM. The effects are considered as reflecting repeated exposure toxicity and not just acute toxicity. Because they occurred within the range of guidance values (CLP Guidance, Table 3.9.2-a, ≤100 mg/kg bw/d for an oral 90-day study) and the effective dose is considerably lower than the acutely toxic dose, it should be classified for STOT RE. Local effects in the gastrointestinal tract (such as chronic oesophagitis, gastritis) after repeated/prolonged exposure are toxicogically relevant as they impair not only the morphology and/or function of the locally targeted organ, but also bear the potential to impair adherent tissues/organs by transmural extension of the chronic inflammation (e.g. peritonitis, pleuritis) or to cause delayed mortalities (after ulceration into body cavities). Thus, RAC agrees to classify MBM as STOT RE 2, H 373 - May cause damage to (gastrointestinal tract) through prolonged or repeated exposure.

Dermal route

Morpholine

No guideline-conforming repeated dose study using the dermal route is available. Only limited information is available from a study published in 1939 describing repeated dermal exposure of rabbits to morpholine diluted with 2 parts of water (33% solution) at a dose level of 900 mg/kg bw/d, which resulted in death of all 7 animals before the 11th dose (cf. MBM_Doc III App. Morpholine) . It is stated that the skin was necrotic, having a thickened oedematous area under the application site; the underlying organs showed inflammation and congestionIn contrast, only a thickening of the skin, but no relevant effects, were detected with morpholine (using sulphuric acid for neutralization) applied to 3 guinea pigs after 30 daily applications (Shea, 1939)..

With regards to systemic toxicity after repeated dermal exposure, no information is given from these early studies.

Formaldehyde
No valid dermal repeated dose study seems to be available (see core document on formaldehyde). There are several long-term studies with an unusual treatment regimen (twice weekly for 60 wks, thrice weekly for 26 wks, 2-3 weeks with documentation on the application frequency) on formaldehyde at concentrations of 0.1% to 10% that revealed mild to moderate irritation from concentrations of 0.5% onwards. Whether systemic effects were examined in these studies, is neither documented in the CLH report nor in the core document on formaldehyde.

MBM
No repeated dose study using the dermal route is available.

Taking the data from formaldehyde into account and the fact that reliable studies on MBM (and morpholine) are not available, the overall database is not sufficient to conclude on classification for STOT RE for this route.

Inhalation route

Morpholine
Irritation of the eyes and nasal cavity: nasal cavity with infiltrates, metaplasia and necrosis, but no systemic effects were observed in a 104 week study in rats exposed to 181 mg/m³ morpholine and in rats exposed to 900 mg/m³ for 7 or 13 weeks (MBM_Doc III App. Morpholine). Considering the exposure duration and the effect levels (in comparison to Table 3.9.2-a in CLP Guidance) no classification for morpholine is warranted.

Formaldehyde
Due to the lack of data on MBM, data on formaldehyde were assessed for STOT RE:

Classification on effects from repeated inhalation exposure may be considered if doses are much lower than those that induce acute irritant or corrosive effects.

As explained for the oral route, CLP Guidance does not say that effects on tissues along the administration routes resulting from repeated exposures are covered by classification for skin corrosion, while it gives some recommendation in Annex I 3.9.1.6, when STOT SE might be more appropriate than STOT RE:

> *Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.*

In addition, Section 3.9.2.5.1 gives guidance on the relevant doses

> *Substances (or mixtures) classified as corrosive may cause severe toxicological effects following repeated exposure, especially in the lungs following inhalation exposure. In such cases, it has to be evaluated whether the severe effect is a reflection of true repeated exposure toxicity or whether it is in fact just acute toxicity (i.e. corrosivity). One way to distinguish between these possibilities is to consider the dose level which causes the toxicity. If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity.*

In short, if doses are considerably lower than those being acute toxic/irritant and these

low doses induce serious health damage after repeated inhalation with accumulation/ exacerbation of repeated insult, classification for STOT RE should be considered.

For formaldehyde, the acute inhalation LC_{50} was reported to be 0.6 mg/L (600 mg/m³) by Nagorny et al. (1979) (see Formaldehyde Core Document II, Table 3-2). Taking the AEC of 0.12 mg/m³ from human data into account, the surrogate effect for repeated inhalation toxicity occurs at 5000-fold concentrations below the acute toxic dose, thus indicating that a classification for repeated inhalation effects is warranted.

There are no human data that examined chronic non-neoplastic lesions in the respiratory tract in humans under controlled exposure conditions. Instead, existing limit values were derived from surrogate data on sensory irritation effects on eyes, nose and throat as this effect is considered as the most sensitive adverse (non-neoplastic) effect. SCCS (2014) in their evaluation considered eye irritation as the most sensitive effect:

> *Eye irritation was revealed as most sensitive adverse endpoint. In susceptible individuals, slight discomfort due to eye irritation occurred at 0.25 ppm but dose-dependent increases in eye irritation were not observed below 1 ppm.Objective ratings for eye irritation (conjunctival redness and eye blinking frequency) have been investigated in healthy volunteers and a NOAEL of 0.5 ppm (without exposure peaks) and 0.3 ppm (with exposure peaks of 0.6 ppm) was established.*

However data on sensory irritation can not be used to decide on classification for chronic toxic effects.

Repeated inhalation studies in animals reported dose-dependent non-neoplastic lesions in the nasal cavity that increased in severity and extent with exposure time and dose (for review see SCCS, 2014; BfR, 2006). Following inhalation exposure up to 24 months, squamous metaplasia was observed in rats at 6 ppm formaldehyde. Epithelial hypertrophy, hyperplasia and metaplasia, mixed inflammatory cell infiltrates and turbinate adhesions were seen at 10 ppm; in addition destruction of turbinate architecture occurred at 15 ppm (Monticello et al., 1996, cited from BfR, 2006). While lesions of the respiratory epithelium in the nasal cavity were not reported after 6 weeks exposure up to 2 ppm (Monticello et al., 1991; Formaldehyde Core document IIIA), inhalation exposure of ≥12 months to ≥2 ppm (2.456 mg/m³) formaldehyde caused purulent rhinitis, epithelial dysplasia and squamous metaplasia at level I of the nasal cavity (Kerns et al., 1983 a, b, cited from BfR, 2006). At higher concentrations than 2 ppm, lesions extended to more posterior parts of the nose (level I to III) and reached the trachea at 14.3 ppm. Monticello (1989, cited from RAC Opinion on formaldehyde) has demonstrated that inhalation of 6 ppm formaldehyde for 1 or 6 weeks induced loss of cilia, inflammatory response, epithelial hyperplasia and squamous metaplasia and increased cell proliferation in the nasal passages of rhesus monkeys. Like in rats, lesions in monkeys showed an anterior-posterior gradient and a duration-related increase in severity and extent of lesions, but these were more widespread than in rats. Inhalation of 3 ppm formaldehyde over 26 weeks induced squamous metaplasia and hyperplasia in the nasoturbinates in 6/6 Rhesus monkeys, but no effects were observed at 0.2 and 1 ppm (Rusch et al., 1983, see SCCS, 2014).
Taking 2 ppm formaldehyde as a robust LOAEC for chronic inflammatory and meta/hyperplastic lesions secondary to initial cytotoxicity in the nasal mucosa from repeated/prolonged inhalation and using the standard Haber's rule extrapolation from 12-month to 90-day exposure to compare with the guidance values, 2 ppm for 12 months corresponds to 8 ppm (9.824 mg/m³ = 0.01 mg/L) after 90 days. This is clearly below the guidance concentration for gases of 50 ppm and would justify a classification of formaldehyde as STOT RE 1.

<u>MBM</u>
No repeated dose study using the inhalation route is available.

The DS suggested read across to the hydrolysis product formaldehyde on which a local inhalative AEC of 0.12 mg/mg³ was based on human data on eye irritation.

Referring to the CLP Regulation Section 3.9.2.10.3, RAC agrees with the DS on that data from formaldehyde may be used, as data on repeated inhalation toxicity of MBM are lacking. However RAC does not agree that effects from repeated inhalation are covered by the classification for corrosion.

The absence of an entry on formaldehyde for STOT RE in CLP, Annex VI does not by itself justify non-classification for MBM.

It was noted in the CLH report that MBM contains about 16.7% releasable formaldehyde. Assuming that under prolonged inhalation exposure conditions MBM would continuously release the maximal releasable amount of MBM%, a factor of 6 should be applied to correct for the lower content of formaldehyde. As the human AEC was based on eye irritation, an acute receptor-mediated sensory irritation effect (without obvious cytotoxicity and infiltration of inflammatory cells) as surrogate for the lowest adverse effect in humans, animal data on repeated inhalation toxicity may be more appropriate to conclude on the classification for STOT RE.

For MBM, the LOAEC for repeated inhalation exposure is based on the LOAEC of 2 ppm for formaldehyde (2.456 mg/m³, derived from a rat 12-month study; Kerns et al., 1983 a,b) (corresponding to 8 ppm (9.824 mg/m³ = 0.01 mg/L)/90-day inhalation based on Haber's rule), corrected for the maximal amount of releasable formaldehyde (16.7%) from MBM with a factor of 6 and reveals a (corrected) concentration of 0.06 mg/L for MBM which is below the guidance value (for mists) for STOT RE 2 (≤0.2 mg/L). As inhalation exposure to the aerosol is expected to be the main concern for MBM, the guidance values for the gaseous form were not considered.

If the chronic toxicity occurred at the same dose level as the acute inhalation toxicity, chronic toxicity would be covered by the classification for acute toxicity. The inhalative LC_{50} was unknown for MBM as no acute inhalation study is available. As a substitute, information on the difference between the level of the inhalation LC_{50} and the LOAEC for chronic effects for formaldehyde was considered. The Formaldehyde Core Document indicates an LC_{50} of 0.6 mg/L (4 h) which is markedly higher than the LOAEC for chronic effects (2 ppm = 2.456 mg/m³). Thus the acute toxicity classification does not cover the classification for STOT RE.

Repeated inhalation exposure to MBM generates the hydrolysis products formaldehyde and morpholine. That morpholine may exert additive effects to those expected from formaldehyde may be expected (as repeated inhalation induced irritant effects to the respiratory tract), however its quantitative impact remains unknown.

Based on the data on formaldehyde (see above), RAC agrees to classify MBM with regards to target organ toxicity from repeated inhalation as **STOT RE 2**.

All routes/Overall classification on STOT RE
If classification for STOT RE is proposed based on data from several routes with different target organs, the final labelling should consider all the relevant target organs. RAC agrees that classification of MBM is warranted as **STOT RE 2, H373: (May cause damage to the respiratory tract and the gastrointestinal tract through prolonged or repeated exposure)**.

No specific route should be indicated.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

4.8.1.1 In vitro data - MBM

Table 4.8-1 Genotoxicity of N,N'-methylenebismorpholine in vitro

Test system Method Guideline	organism/ strain(s)	concentra-tions tested	Result		Remark	Reference
			+ S9	- S9		
Salmonella microsome assay, OECD 471	S. typhimu-rium TA98, TA100, TA1535, TA1537; E. coli WP2uvrA$^-$	5, 15, 50, 150, 300, 500, 1000, 1500, 5000 µg/plate	(+)	-	Only in TA100 a reproducible slight increase in revertants detected with S9-mix at a non-cytotoxic dose level and which was above the historical control range (still below 2 fold above actual control).	Lubrizol (2000) MBM-Doc III A6.6.1
Chromosome aberration test; OECD 473	Chinese hamster lung (CHL) cells	0, 3.75, 7.5, 15, 30, 45, 60 µg/ml with-out S9 and 0, 7.5, 15, 30, 45, 60, 90, 120 µg/ml with S9	+	+	The test substance induces with and without MA dose dependent increases in aberrations even at non-cytotoxic concentrations. Induces also polyploidy.	Lubrizol, 2001; MBM-Doc III A6.6.2
Mouse lymphoma assay; OECD 476	Mouse lymphoma L5178Y TK+/- 3.7.2c cells	0, 3.75, 7.5, 15, 30, 45, 60 µg/ml	+	+	Considered to be mutagenic with and without MA; predominantly clastogenic activity	Lubrizol, 2001; MBM-Doc III A6.6.3

(+): weak positive; MA: metabolic activation

In the Salmonella microsome assays weak mutagenic activity was reported at 300 µg/plate with metabolic activation in TA100 (cf. MBM-Doc III A6.6.1).

In a valid chromosome aberration assay on CHL cells clastogenic activity was demonstrated with and without metabolic activation. The chromosome mutagenic effects were detected even at non-cytotoxic dose levels (cf. MBM-Doc III A6.6.2).

Accordingly, positive results were reported in a study on gene/chromosome mutations in the mouse lymphoma assay. An increase in mutation frequency was observed independent on metabolic activation system. The test substance induced more small than large colonies indicating predominantly clastogenic activity. (cf. MBM-Doc III A6.6.3).

In summary, the in vitro results on genotoxicity revealed evidence for mutagenic activity.

4.8.1.2 In vivo data - MBM

Table 4.8-2 Genotoxicity in vivo

Type of test Method/ Guideline	**Species Strain Sex no/group**	**Frequ-ency of appli-cation**	**Samp-ling times**	**Dose levels**	**Results dose, sampling time and result +/-/± dose x, sampling time y:**	**Remarks**	**Reference**
Mouse bone marrow mic-ronucleus test; OECD 474	Mouse ICR 7 m	Single oral appli-cation	24 and 48 h after applica-tion	0, 250, 500, 1000 mg/kg bw (0, 2.5, 5, 10% in oil)	250 mg/kg bw, 24 h: - 500 mg/kg bw, 24 h: - 1000 mg/kg bw, 24 h: - 1000 mg/kg bw, 48 h: -	Valid, MTD reached	Lubrizol (2001) MBM-Doc III A6.6.4
Unscheduled DNA syn-thesis (UDS) in rats; OECD 486	Rat Sprague-Dawley 4-6 m	Single oral appli-cation	Perfu-sion 16 h or 2 h af-ter app-lication	0, 300, 900 mg/kg bw (0, 3, 9% in oil)	300 mg/kg bw, 2 h: - 300 mg/kg bw, 16 h: - 900 mg/kg bw, 2 h: - 900 mg/kg bw, 16 h: -	Valid UDS in hepatocytes	Lubrizol (2002) MBM-Doc III A6.6.5

MBM was tested in the mouse bone marrow micronucleus test (cf. MBM-Doc III A6.6.4). The test substance did not induce a significant increase in the number of micronuclei at a dose level up to 1000 mg/kg bw. The high dose induced cytotoxic effects in the bone marrow. This might be a systemic effect but it is not excluded that these effects are secondary to the local effects (unfortunately no necropsy data available) in the gastro-intestinal tract.

MBM did not induce unscheduled DNA synthesis in the liver at a dose levels up to 900 mg/kg bw, the maximum tolerated dose. At this dose level hunched posture, noisy respiration, and diuresis were observed and might represent systemic effects but it is not excluded that these effects are due to local effects (unfortunately no necropsy data available) in the gastro-intestinal tract (Lubrizol, 2001; cf. MBM-Doc III A6.6.5).

4.8.2 Human information - MBM

Not available.

4.8.3 Products of hydrolysis – in vitro

Formaldehyde (Formaldehyde – Doc II A3.6.1 & Formaldehyde – Doc III A6.6)

Mutagenic and clastogenic activity of formaldehyde in vitro is well documented. Positive results in Ames tests in S. typhimurium strains TA97, 98, 100, 102 and 104 suggest that formaldehyde may induce mutations by various mechanisms and independent of metabolic activation (Haworth et al., 1983; Marnett et al., 1985). TK and HPRT gene mutation analysis in mammalian cells confirmed this activity following exposure to ≥ 3 µg/mL for 4 hours or ≥ 9 µg/mL for 1 hour, respectively (Blackburn et al., 1991; Grafstöm et al., 1993). A mechanistic study by Liber et al. (1989) suggested that mutagenesis by formaldehyde in mammalian cells involves base pair substitutions as well as deletions. This is in accordance with induction of single strand breaks and DNA crosslinks reported following treatment with ≥ 6 or 3 µg/mL formaldehyde for 90 min, respectively (Cosma & Marchok, 1988) and sister chromatid exchange, micronuclei formations and DNA crosslinks observed at ≥ 1.9, 3.8 or 3.89 µg/mL over 4 hours, respectively (Merk & Speit, 1998). In these studies, time-dependent repair of the lesions was also reported. Nevertheless, formaldehyde proved clearly clastogenic at concentrations ≥ 16 µg/mL x 8 h in CHO Chinese hamster ovary cells and at ≥ 7.5 µg/mL x 1 h in human primary lymphocytes (Galloway et al., 1985; Schmid et al., 1986). Addition of S9-mix for metabolic activation did generally not enhance the genotoxicity of formaldehyde, but may support detoxification in some cases (Haworth et al., 1983; Galloway et al., 1985; Schmid et al., 1986; Blackburn et al., 1991).

Morpholine (Morph-Doc III A6.6.1 & Morph-DocIIIA6.6.3 & Morph-DocIIIA6.6.1-6)

Sufficient data are available on the bacterial reverse mutation assay. At dose levels up to 5 mg/plate, the max. concentration recommended in OECD guideline 471, no mutagenic activity was detected. A slight increase in revertants was found only at extremely high doses > 5 mg/plate. No data were given on pH or osmotic pressure but these parameters might play a critical role at these dose levels.

In further studies no mutagenic activity was detected in S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation at concentrations up to 10 mg/plate.

Data on the endpoint clastogenicity in vitro are lacking. In the mouse lymphoma assay no conclusive indication for mutagenic effects without metabolic activation was reported, with metabolic activation no mutagenic activity was found. Studies on additional endpoints like gene conversion in fungi, DNA damage, SCE or cell transformation in mammalian cells gave no clear evidence for genotoxicity.

4.8.4 Products of hydrolysis – in vivo

Formaldehyde (Formaldehyde – Doc II A3.6.2 & Formaldehyde – Doc III A6.6.4-6)

Although formaldehyde is locally genotoxic in somatic cells at the site of contact, the presently available data supports the conclusion that germ cells are not affected. Therefore, labelling for mutagenicity according to Directive 67/548/EEC or EU Regulation 1272/2008/EC is not required.

Morpholine (Morph-Doc II A4.6.2 & Morph-Doc III A6.6.1-6 Add Info)

In the comet assay (detection of DNA damage) ddy mice received 0 or 600 mg/kg bw (1/2 of LD50) and were sacrificed 3 or 24 h after treatment. Stomach, colon, liver, brain, bone marrow, kidney, bladder, and lung were studied in the alkaline single cell gel electrophoresis. The concentration of morpholine in vehicle (no details) was high enough to damage the stomach mucosa. But no significant DNA migration was observed in any organ suggesting no DNA damage in mice after oral application of 600 mg/kg bw.

Further studies on genotoxicity of morpholine in vivo are available with mainly negative outcome (host mediated assay, negative; transplacental clastogenic or gene mutagenic effects in hamster embryos, negative; clastogenic effects in the rat, positive). However, the validity of these assays is insufficient for evaluation.

In conclusion, the available data in vitro and in vivo indicated that morpholine has no genotoxic properties.

4.8.5 Comparison of MBM with the hydrolysis products

Table 4.8-3 Comparison of data on genotoxicity in vitro

Parameters	**MBM**	**Formaldehyde**	**Morpholine**
Gene mutation in bacteria	Weak mutagenic	Mutagenic	Negative
Chromosome aberration in eukaryotic cells	Clastogenic > 30 µg/ml	Clastogenic, > 7.5 µg/ml	No data
Gene mutation in mammalian cells	Mutagenic, predominantly clastogenic	Mutagenic	Negative
DNA damage in bacteria and eukaryotic cells	No data	Genotoxic	Negative
Overall assessment	Mutagenic activity in vitro	Mutagenic activity in vitro	No mutagenic activity in vitro

Table 4.8-4 Comparison of data on genotoxicity in vivo

Parameters	**MBM**	**Formaldehyde**	**Morpholine**
Systemic genotoxicity	**Negative** (mouse bone marrow micronucleus assay & UDS assay in rats)	**Negative** (cytogenetic & micronucleus assay) contradictory results in humans	Negative (comet assay:liver, brain, bone marrow, kidney, bladder, lung)
Local genotoxicity	**No data**	**Positive** (clastogenic in the gastrointestinal tract of rats after oral exposure; clastogenic in the upper respiratory tract of humans after inhalation; DNA-protein cross-links at the site of first contact after inhalation exposure)	Negative (comet assay: stomach, colon)

4.8.6 Summary and discussion of mutagenicity

In vitro studies on MBM gave some evidence for gene and clear evidence for chromosome mutagenic activity in vitro. It is considered that the genotoxicity is related to the hydrolysis product formaldehyde which is formed in the aqueous test solution for the in-vitro genotoxicity tests. The DNA-protein cross-linking activity of formaldehyde is a possible mechanism. Genotoxicity tests for morpholine result overall negative.

In vivo MBM has no systemic clastogenic activity in the mouse bone marrow micronucleus assay and did not induce DNA damage in rat hepatocytes in the UDS assay. These assays are not suitable to detect local genotoxicity of MBM in vivo. Data on the hydrolysis product formaldehyde suggested more local than systemic mutagenic effects. Formaldehyde is genotoxic in vitro and in vivo it induces local clastogenic effects. Similar results are expected for MBM. The hydrolysis products morpholine did neither show mutagenic in vitro nor in vivo systemic nor in vivo local.

4.8.7 Comparison with criteria

Based on the available data and mechanistic considerations of formaldehyde release local genotoxic effects are to be expected from MBM. The presently available data for MBM, FA and Morpholine support the conclusion that germ cells are not affected and according to CLP Regulation 1272/2008/EC, Annex 1, paragraph 3.5.2.1 the germ cell mutagenicity "hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny." However according to the ECHA CLP guidance 2012, chapter 3.5.1 "genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, "site of contact" genotoxicants)" may be classified as category 2 mutagen in order to provide an indication that the substance could be carcinogenic. Nevertheless, since the substance is already proposed for classification as carcinogenic Cat 1B, it was considered that there is no need for this further information and labeling for mutagenicity according EU Regulation 1272/2008/EC should not be required.

However during RAC meetings for the classification of formaldehyde (2012), the hazard classes on mutagenicity and their interpretation with regard to the classification of somatic cell mutagenicity were discussed on a very fundamental level. RAC agreed that "due to the induction of genotoxic effects in vivo on somatic cells at site of contact, which are supported by positive findings from mutagenicity and genotoxicity tests in vitro, … classification of formaldehyde for mutagenicity category 2 in accordance with the CLP Regulation, with the hazard statement H341 (Suspected of causing genetic defects) is therefore warranted. The route(s) of exposure should not be stated in the hazard statement as it is not proven that other routes than inhalation can be excluded."

It is proposed to base classification of MBM on the data of the hydrolysis product formaldehyde. Arguments for and against reading across the carcinogenicity data and C&L conclusion from

formaldehyde to MBM are listed in chapter 4.9.5. The same arguments are valid for the read across of mutagenicity category 2. A consistent approach for the read across for these 2 endpoints is necessary.

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4.8.8 Conclusions on classification and labelling

Classification for mutagenicity, category 2 is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The DS proposed to classify MBM as a Caterory 2 mutagen based on the existing classification of its hydrolysis product formaldehyde.
There are several mutagenicity studies *in vitro* and *in vivo* for MBM that were considered valid. Predominantly clastogenic effects were induced in cells of mammalian cell cultures with and without metabolic activation; a bacterial gene mutation test was weakly positive only with metabolic activation. Regarding the *in vivo* testing, a negative micronucleus test and a negative UDS test were available.

The DS additionally provided information on negative results of *in vitro* mutagenicity tests and *in vivo* genotoxicity tests for the hydrolysis product morpholine.

The DS argued that due to the hydrolysis of MBM to fromaldehyde at contact to biological tissues, induction of local genotoxic effects is to be expected at site of first contact *in vivo*. Therefore the DS refered to the existing classification of formaldehyde as a Category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at site of contact which are supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests.

Due to the mechanistic considerations of formaldehyde release from MBM the applicant proposes to classify the substance MBM as a Category 2 mutagen on the basis of its hydrolysis product formaldehyde.

Comments received during public consultation

Two Member States expressed their support for the proposed classification. One individual comment disagreed with the proposed classification as a Category 2 mutagen due to the lack of relevant mutagenicity data.

Assessment and comparison with the classification criteria

<u>Formaldehyde</u>
RAC agrees with the approach of the applicant to take into account the classification of formaldehyde as Category 2 mutagen for justification of the classification of MBM.

Formaldehyde that is released from MBM at contact with biological tissues is classified as a Category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at the site of contact which are supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. Although it seems likely that the amount of formaldehyde may vary depending on different uses, the inherent potential of MBM to release formaldehyde is a critical fact.

Testing of the *in vitro* mutagenicity of MBM shows that the observed positive effects are consistent with those known from formaldehyde alone. It is assumed that MBM has a low systemic availability *in vivo* due to its hydrolysis. Therefore it seems to be unlikely that

genotoxic effects are induced at a site distant from first contact.

Information on the hydrolysis product formaldehyde was used to assess the mutagenic potential of MBM.

Morpholine
RAC takes note of the additional information by the applicant that no indication for *in vitro* mutagenicity and *in vivo* genotoxicity of the hydrolysis product morpholine has been detected in available *in vitro/in vivo* studies and no relevant structural alerts are present.

MBM
The evaluation of the mutagenicity data of MBM by the DS and RAC do are in agreement. RAC also comes to the conclusion that a proposal for classification of MBM as a Category 2 mutagen is justified.

In vitro data
The available bacterial gene mutation test is weakly positive with S9-mix (Lubrizol (MBM-Doc III A6.6.1), 2000).

A mouse lymphoma assay (Lubrizol (cf. MBM Doc III A6.6.3), 2001) is positive with and without S9-mix. At the analysis of the colony sizes, predominantly small colonies were found, which indicates clastogenic activity of MBM.
A chromosomal aberration test is positive in CHL cells with and without S9-mix (Lubrizol Corporation (cf. MBM Doc III A6.6.2), 2001).

In vivo data
An *in vivo* mouse bone morrow micronucleus is negative after single oral application up to the highest tested dose of 1000 mg/kg bw (Lubrizol (MBM Doc III A6.6.5), 2001). It was reported that the highest tested dose induces cytotoxic effects; necropsy data were not available.
An *in vivo* UDS test with rats is negative after single oral application of 300 and 900 mg/kg bw (Lubrizol (MBM Doc III A6.6.5), 2002). After the application of the highest tested dose clinical signs were observed; necropsy data were not available.

The quantity of test data for MBM is limited and the mutagenicity studies are not published. Thus, only the data given by the DS are available. These data allow neither a detailed test evaluation nor do they allow to conclude on whether a test performance is fully in accordance with the corresponding guideline. Despite these limitations, the following conclusion can be drawn: In bacteria as well as in soma cell cultures mutagenic effects are induced. The results of an *in vivo* micronucleus test as well as an *in vivo* UDS test are negative.

RAC considered that due to its reactivity, a low systemic availability is expected for MBM and therefore the induction of systemic genotoxic effects is unlikely. However, a local genotoxic effect produced by the hydrolysis product formaldehyde is expected and RAC considers that use of data from formaldehyde, which is classified as a mutagen Cat. 2 based on its local genotoxic action, is justified. For information regarding the induction of local effects at the sites of contact after repeated oral dosing (gavage) or repeated inhalation of MBM see point '4.7. Repeated dose toxicity' of the CLH report.

Some RAC members expressed their view that the guidance relates only to classification of substances that caused germ cell mutations. This view is reflected in a minority position supported by three RAC members. RAC recognised that according to CLP Guidance, Section 3.5.1, classification is also warranted if there is evidence of only somatic cell genotoxicity that leads to classification in Category 2 if genotoxic substances are only acting locally.

RAC agrees with the proposal of the DS to classify MBM as a Mutagen, Category 2 H341 (Suspected of causing genetic defects) based on relevant data from 'its hydrolysis product formaldehyde.

Supplemental information - In depth analyses by RAC

Analyses

According to the CLP Guidance, hazard classification for germ cell mutagenicity primarily aims to identify substances causing heritable mutations in germ cells or suspected of causing heritable mutations due to the induction of genotoxic effects in soma cells *in vivo*. This applies for substances with sufficient systemic availability. In addition, information is given whether it is possible that genotoxic effects may play a role in carcinogenesis. Therefore the guidance also recommends a possible classification of substances that can act only locally in soma cells at site of first contact due to their poor systemic availability.

It is assumed that MBM has a low systemic availability due to its reactivity. Accordingly, the available *in vivo* results are of low relevance and do not allow the conclusion to be drawn that the substance is not genotoxic in the whole animal. There is no test with MBM which assessed whether genotoxic effects will be induced in cells at site of first contact. But for the evaluation of the toxicological properties of MBM it is taken into account that its hydrolysis product formaldehyde is already classified as Category 2 mutagen due to the induction of local genotoxic effects.

4.9 Carcinogenicity

4.9.1 Non-human information - MBM

No long-term carcinogenity study on experimental animals is available for MBM.

4.9.2 Human information - MBM

No human data are available for MBM.

4.9.3 Products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.7; Formaldehyde – Doc III A6.7 & - Doc III A6.12.4)

In conclusion, experimental evidence in rats and mice demonstrates that long-term formaldehyde gas inhalation causes tumours in the upper respiratory tract from exposure concentrations of 7.2 µg/L. The relevance of this effect for human health was recently confirmed by an independent assessment within the IPCS Human Framework for Analysing the Relevance of the Cancer Mode of Action for Humans (McGregor et al., 2006).

Taking into account the dose-response after subacute, subchronic and chronic inhalation exposure, it can further be concluded that the threshold dose for local lesions remains practically constant with increasing time, while the nature of the lesions reflects the progressing pathology (see 3.5). Hence, an overall inhalation NOAEC of 1.2 µg/L for local effects based on the 6-mo studies in rats and monkeys is derived (Rusch et al., 1983, see also section 3.5).

Formaldehyde shall be classified as Category I carcinogen, "May cause cancer by inhalation", H350.

Morpholine (EG – Doc II A4.7; EG – Doc III A6.7)

Results in a long-term drinking water study in B6C3F1 mice did not show any carcinogenic effects of MOAS (morpholine oleic acid salt). No increased incidences in any tumour type were reported in a long-term inhalation study in Sprague-Dawley rats. These results are in accord with data presented on genotoxicity of morpholine which indicated no mutagenic properties.

4.9.4 Comparison of MBM with products of hydrolysis

Table 4.9-1 Comparison of data on carcinogenicity

Parameters	**MBM**	**Formaldehyde**	**Morpholine**
Systemic carcinogenicity in experimental animals	No data	No carcinogenic activity	No carcinogenic activity
Local carcinogenicity in experimental animals	No data	Carcinogenic activity after inhalation at ≥ 7.2 mg/m³. Local promoting activity	No carcinogenic activity
Systemic carcinogenicity in humans	No data	Conflicting results, biologically not plausible	No data
Local carcinogenicity in humans	No data	Carcinogenic activity in respiratory tract	No data

4.9.5 Summary and discussion of carcinogenicity

In summary it is considered that the equilibrium of MBM and formaldehyde shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media. This assumption is –in qualitative terms- supported by the hydrolysis study and the intratracheal kinetic study. Furthermore the tests for systemic genotoxicity were negative for MBM. The hydrolysis products formaldehyde and morpholine are unlikely to induce systemic genotoxicity or carcinogenicity as demonstrated by respective negative genotoxicity tests and carcinogenicity studies.

Consequently it is to be expected that MBM shows the same local carcinogenic hazard as Formaldehyde.

The following options are considered for decision on classification and labelling: In the situation when the concentration of formaldehyde in the formaldehyde releasing substance is equal or higher than the general classification limit (0.1% in case of GHS class 1, 1% in case of GHS class 2) the classification should be the same as the classification established for formaldehyde. However, when the concentration will be lower than the general classification limit in principle two options may be followed:

(I) The formaldehyde releasing substance should be classified like formaldehyde - based on the considerations of total releasable formaldehyde, intended use, category of users and exposure taking into account the precautionary principles in this case of difficulties with the risk assessment of substances that are instable, showing equilibrium behaviour and having half lives depending on dilution, temperature and/or UVCB characteristics.

(II) The formaldehyde releasing substance should be classified one class higher (GHS class 2) of that for formaldehyde or not classified in case formaldehyde will be classified in GHS class 2 – based on the formal consideration as constituent of a product at the time being "supplied to the user".

Below the arguments for both of the options are summarized:

Table 4.9-2 Arguments for classification of MBM based on "total releasable formaldehyde" or "free formaldehyde" content

supportive arguments for proposal 1:	**supportive arguments for proposal 2:**
Classification according to releasable Formaldehyde, i.e. Skin Corr. 1B, Skin Sens 1, Carc. 1B	Classification according to "free Formaldehyde", i.e. Skin Corr. 1B
Risk through formaldehyde-release in water is covered According to CLP Regulation Annex I, paragraph 1.1.1.3 a WoE evaluation is required for	Classification usually relates to the substance itself and not to potential release or degradation products which occur during different use scenarios

classification and labelling purposes including "information on substances or mixtures related to the substance or mixture being classified".	
The formaldehyde releaser is difficult to characterise since it shows equilibrium behaviour and having half-lives depending on dilution, temperature and pH.	Analogue to the evaluation of other "substances of concern" or impurities the cut-off values from the GHS system should be considered for the real amount of free formaldehyde
If classification considers the handling, the dilution and the release kinetics should be considered as well: The DT50 of the release was measured as 2.4 hours at 50°C and probably also at 37°C (study documentation is limited for the latter). Each mg MBM releases 0.16 mg formaldehyde	Formaldehyde -releasers are designed as transport forms and depot compounds and these benefits of slow continuous formaldehyde release should be considered. Formaldehyde releasers should not be equalized with a pure formalin-solution.
Formaldehyde release is a hydrolysis and occurs with contact with biological tissue and media	
Solutions of formaldehyde releasers only need to be classified if formaldehyde content is above 0.1%	Formaldehyde release is a hydrolysis and occurs in dilutions with water → depending on the releaser type this needs dilutions between 1:10 and 1:1000
In vitro genotoxicity data for MBM support the assumption of <u>local</u> genotoxicity and consequent <u>local</u> carcinogenicity	Other examples for substances (oligomers) that contain formaldehyde and are classified according to free formaldeyhde: ● Polyoxymethylen (CAS formaldehyde-polymer = technical plastic) has different properties compared to FA and is classified differently ● Paraformaldehyde itself (degree of polymerization of 8–10 units) is only classified as toxic (T) and corrosive (C) so far Instead of full classification and labelling a warning label could be applied „can release FA with water contact" A classification of formaldehyde-releasers on the basis of maximal releasable formaldehyde could be considered as an unusual mixture between the classification process and risk assessment which does not justify either of the both procedures

A third possibility may be to classify the formaldehyde releaser in Carcinogenicity category 2 in order to account for the uncertainties for substances that are instable, showing equilibrium behaviour and having half-lives depending on dilution, temperature and pH.

The applicant summarized the following consequences of classification according to maximal releasable formaldehyde (proposal 1):

- Classification and labelling implies a lot additional requirements for storage and transport
- High protection measures need to be implemented (e.g. respiratory protection at refilling) also in cases where only a low risk is existent (no water contact)
- Possible products and uses will be impossible on the market due missing users acceptance (panics); as a last consequence a whole group of substances showing a high and broad efficacy could disappear from the market and will be replaced by other products showing other problems which presumably do not have a comparable efficacy

4.9.6 Comparison with criteria

Genotoxiciy data for MBM support local genotoxicity, but no systemic genotoxicity. No carcinogenicity studies are available for MBM. However carcinogenicity data available for the hydrolysis product formaldehyde support classification for category 1B on the basis of human and animal data. Formally "information on substances or mixtures related to the substance or mixture being classified" should be used within a WoE evaluation for classification and labeling. It is proposed to base classification of MBM on the data of the hydrolysis product formaldehyde. Arguments supporting classification in Category 1B and arguments for non-classification are listed above.

4.9.7 Conclusions on classification and labelling

Classification for carcinogenicity, category 1B is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No cancer bioassay or human data were available for the substance. The DS proposed classification for carcinogenicity based on data for formaldehyde.

Comments received during public consultation

Four MSCA supported the classification for carcinogenicity. Industry argued that classification is not warranted.

Assessment and comparison with the classification criteria

Morpholine

No carcinogenic effect was observed in a combined chronic toxicity and carcinogenicity drinking water study (reported as comparable to OECD TG 453, only two test doses) in mice on MOAS (morpholine oleic acid salt). Shibata et al.(1987a) (see MBM_Doc III App. Morpholine) exposed 50 male and 50 female B6C3F1 mice to MOAS for 96 weeks followed by a post-observation period of 8 weeks via the drinking water containing 0, 0.25% and 1.0% MOAS (equivalent to 0, 400 and 1500 mg/kg bw/d, respectively, in males and 0, 500 and 1500 mg/kg bw/d, respectively, in females; 0.25% and 1% MOAS corresponded to 0.06% and 0.24% morpholine). In high dose mice, reduced body weight gain, water intake, increased blood urea nitrogen (males only) and increased incidences of squamous hyperplasia of the forestomach) were observed. At the low dose, lower body weight gain was seen in female mice.

In a 104-week inhalation study in Sprague-Dawley rats (57-60 animals/sex/group), the animals were exposed to 0, 10, 50, 150 ppm morpholine vapour (0, 36, 181, 543 mg/m³, respectively), 6 h/d, 5 d/wk (Harbison et al, 1989, see App. Morpholine). Local irritation of eyes and nares, chromodacryorrhea and urine stains of the fur were observed at 150 ppm. Similar effects in a few mid dose males and females were reported in the study summary and it was noted that no details were given and the reported incidence was found questionable. The RMS added that the grossly observed effects exhibited as localized sores, bloody crust about the eyes, nose, face and body, localized necrosis (skin) and chromodacryorrhea and urine stains at the 181 and 543 mg/mm³ doses. A concentration-related increase in necrosis, infiltration of neutrophils and metaplasia of the nasal turbinates were observed at ≥50 mg/m³. No indication of systemic chronic toxicity or carcinogenicity was detected.

Formaldehyde

The hydrolysis product formaldehyde is classified in CLP, Annex VI for carcinogenicity, Cat 1B.

MBM
There are no long-term/carcinogenicity studies on MBM available.
Human data on MBM are not available (except a summary on a medical data letter report on medical screening results from three workers in the production area).

The non-submission of data was justified by a read across to formaldehyde and probable carcinogenic effects of MBM were considered by the applicant to be related to the hydrolysis product formaldehyde (Doc III A6.7 MBM non sub doc).

RAC considers read-across to the hydrolysis products of MBM justified.
Based on the study summary available (and the identifiable weaknesses of the studies) there was no indication of a tumour response for morpholine from long-term oral studies in mice and from long-term inhalation studies in rats. Carcinogenic potential cannot be totally excluded based on these studies; the squamous hyperplasia of the forestomach seen in oral long-term studies in mice may indicate potential for these to develop into tumours.

Although no concern for a carcinogenic effect has been identified from the available long-tem studies, the effects of morpholine at the site of contact seem to be similar to those of formaldehyde (cytotoxicity, inflammation, metaplasia, hyperplasia in the gastrointestinal tract and the respiratory tract). No information is given on a possible additive contribution to the carcinogenic potential that is derived from the hydrolysis product formaldehyde.

The DS considered that the equilibrium of MBM and formaldehyde shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media. The formaldehyde release is –in qualitative terms- supported by the hydrolysis study, the intratracheal kinetic study, which indicated that formaldehyde is rapidly released, and by the effects at the site of contact observed after repeated oral and inhalation exposure.

The hydrolysis products formaldehyde and morpholine are unlikely to induce systemic genotoxicity or carcinogenicity as demonstrated by respective carcinogenicity studies and negative *in vivo* genotoxicity tests. It is therefore assumed for MBM that, similar to formaldehyde, systemically increased bioavailability and concern for systemic carcinogenic responses are not to be expected.

It is expected that MBM exerts similar effects as formaldehyde such as cytotoxicity, hyperplasia, metaplasia, tumours and local mutagenic effects at the sites of contact – i.e on the epithelium of the respiratory tract, following prolonged inhalation, since formaldehyde is one of the hydrolysis products from MBM.

Formaldehyde is classified based on its carcinogenic potential at the sites of exposure, primarily on the nasopharyngeal tumours observed in man and rodents after prolonged inhalation[5].

CLP Guidance, Section 3.6.2.2.7 states
'A substance that has not been tested for carcinogenicity may in certain instances

[5] http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling?search_criteria_name=Formaldehyde&search_criteria_ecnumber=200-001-8&search_criteria=Formaldehyde

be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes.'

CLP Guidance (Section 1.4.3) explicitly foresees the read across of information from 'source' substances to predict the same hazard for another 'target' substance. For MBM, it is not about the similarity of source and target substance, but MBM should be classified as a carcinogen based on the release of the *identical* substance (formaldehyde) resulting from hydrolytic transformation of MBM.

Endpoints, on which data on MBM are available, show that the similiarity of effects at the site of contact support the use of data from formaldehyde as justified. Similar effects were noted e.g. for the oral repeated toxicity, with the observation that the toxicity may be more severe for MBM when comparing the dose levels or the severity of effects observed with formaldehyde. However uncertainties remain as to the lack of carcinogenicity studies on MBM with full guideline compliance and as to an additional (unknown) contribution of the other hydrolysis product morpholine to the effects by formaldehyde. The kidney was a target organ by repeated exposure to morpholine; the findings in the 14-day range-finding study of pale kidneys and increased kidney weight provide some hint of systemic effects of MBM exposure that may be attributable to absorbed morpholine.

From a quantitative aspect, the hydrolysis rate of MBM to formaldehyde may depend on several environmental factors (temperature, increases at lower pH, and at higher dilutions with aqueous media). Due to a rapid rate of hydrolysis it was not possible to detect MBM at the beginning of measurement and after 2.4 h for the tested pH-levels (37°C for pH 1.2, and at 50°C for pH 4, 7 and 9) (see MBM Doc III A7.1.1.1.1). MBM hydrolysed so quickly that the exact hydrolysis half-life could not be estimated (less than 2.4 h for 50°C). In the view of the RMS, a significantly lower hydrolysis half-life than 1 day at 25°C can be concluded but not quantified based on the present data. The rate may be assumed to be in the range of hours/ minutes.

However, water contact or dilution of MBM with aqueous solutions are not a necessary condition for exerting toxic effects of MBM. For the aerosol, aqueous conditions were given at contact sites (mucous membranes with oral & inhalation exposure, sweaty skin). The CLH report stated that the equilibrium of MBM shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media.

In the public consultation several commenters disagreed with the classification of MBM based on data from formaldehyde and stated that MBM contains one of the lowest levels of total releasable formaldehyde per molecule (16.7%) (*in comparison to other formaldehyde releasers on the market*), less than 0.005% of free (unbound) formaldehyde and that MBM is relatively stable in end use fluids. The release of formaldehyde via volatilisation or MBM by aerosolisation was found negligible and the resulting exposure level at workplaces were not sufficient to cause tumours under conditions of normal use (*in the end products*). Overall, the probability of a carcinogenic potential of MBM was seen as negligible. RAC notes that the CLP Regulation states that classification is based on intrinsic hazards of a substance and does not take the exposure conditions, the exposure to mixtures containing the substance of concern or the anticipated risk level into account.

The option to classify MBM as carcinogen, in category 2, in order to account for uncertainties for substances such as this that are unstable, showing equilibrium behaviour and having variable half-lives depending on dilution, temperature and pH, as discussed as an option in the CLH report is not supported by RAC. By weighing the

evidence from data from the specific substance (and hydrolysis product) that is known to have carcinogenic properties (formaldehyde), no reasons (such as uncertainty about structural similarity or qualititative differences in the mechanistic aspects) could be identified to justify a downgrading of the classification category. Hydrolysis tests demonstrated that formaldehyde is generated within short time periods.
These hydrolysis tests and the intratracheal instillation study support qualitatively that hydrolysis of MBM will occur in contact with aqueous biological media on mucous membranes. Inhalation exposure to aerosolic MBM is expected to result in hydrolysis at the site of contact and toxicologically significant concentrations of formaldehyde could be reached on the surface of the mucous membranes in the respiratory tract, eye or upper GI tract or skin. The inhalation exposure to gaseous formaldehyde that evaporated from MBM is assumed to contribute in (an unknown extent) in addition to the toxic/carcinogenic effect resulting from the direct impact of hydrolysis products at the contact site. Demonstrating that the volatility and the room concentrations of released gaseous formaldehyde will be rather low would not be sufficient to discount the hazardous potential that may result from the inhalation exposure to MBM aerosol.

As no data are available to demonstrate that a sufficiently high concentration of formaldehyde can not (meaning never) be reached, there is no evidence to justify a downgrading. This prerequisite for the evidence is in contrast to the opinion of some commenters who found that the classification is only justified if evidence from exposed workers demonstrates that sufficient formaldehyde will be released and have caused tumours.

Information on the hydrolysis product is used to assess the hazardous properties including the carcinogenic potential of MBM. More guidance is given in REACH, Annex XI, 1.5.2 that specifies that similarities to substantiate the read across may be based on common precursors or common breakdown products via physical or biological processes, which results in structurally similar chemicals.

RAC agrees with the proposal of the DS to classify MBM based on the released formaldehyde as a Carcinogen, Category 1B; H350 (May cause cancer).

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information - MBM

(Summary and Conclusion (MBM-Doc III A6.4.1)

A study on repeated dose toxicity according to OECD 408 in rats has been performed (cf. MBM-Doc III A6.4.1 & MBM-Doc II A4.5). In this subchronic gavage study histophathological examinations included also reproductive organs in males and females. No treatment related effects were observed even at dose levels inducing local effects in stomach and upper respiratory tract. The implementation of a specific reproduction toxicity study is scientifically unjustified because repeated exposure to MBM resulted in local effects but did not affect the reproductive organs.

4.10.1.2 Human information - MBM

No human data available.

4.10.1.3 Products of hydrolysis

Formaldehyde (Formaldehyde - Doc II A3.8.2 & 3.1, - Doc III A6.5.1 and – Doc III A6.8.2)

No fertility studies in animals have been submitted, the epidemiological data on reproductive effects in exposed humans is inconclusive (Collins et al., 2001), and the endpoints assessed in the repeated dose studies summarised above are considered inappropriate to draw conclusions on reproductive toxicity of formaldehyde.

Effects on the male reproductive system including reduced testosterone production, reduced spermatogenesis, impaired sperm function and reduced GSH levels as well as increased rates of sperm abnormalities and elevated malonedialdehyde concentrations were reported in two rat inhalation studies exposing the animals to ≥ 6 or 10 µg/L, indicating that the testis may be a target tissue for formaldehyde toxicity (Özen et al., 2005; Zhou et al., 2006). These findings are consistent with results from studies in rats and mice using intraperitoneal application. The latter uniformly found adverse effects on testicular function above a NOAEL of 0.1 mg/kg bw/d (Majumder and Kumar, 1995; Odeigah, 1997; Tang et al., 2003, Zhou et al., 2006, Zhou et al., 2006b). However, the concentrations at which effects were reported in the inhalation studies have, in other repeated dose studies, influenced body weight gain and food consumption. Unfortunately, a NOAEC was not determined and animals have not been mated to assess effects on fertility. A one-generation feeding study in minks failed to show reductions in litter size despite mild local and systemic toxicity at the top dose of ~ 0.7 mg paraformaldehyde per g food (662 ppm) (Li et al., 1999). Overall, these observations (and the absence of corresponding alerts within the human data) support the general presumption that effects on male reproductive functions may be relevant for inhalative exposure only at higher concentrations concurrent with other local and/or systemic toxicity. This may appear to support the conclusion that systemic effects as described here and in chapters 4.5 and 4.7 might be secondary to local lesions.

Morpholine (Morph-Doc III A 6.8.2 Justification for non-submission)

No studies were available which are directly related to this endpoint (e.g. OECD guideline 415, 416, or 422). In subchronic and chronic inhalation studies in rats (comparable to current guidelines) the reproductive organs were investigated and no adverse effects were found. Accordingly, in a long-term drinking water study in mice exposed to morpholine oleic acid salt also no effects were detected in the reproductive organs.

4.10.1.4 Comparison of MBM with products of hydrolysis

Table 4.10-1 Comparison of data on fertility

Type of study	**MBM**	**Formaldehyde**	**Morpholine**
Repeated dose toxicity (≥ 90 days)	No effects on reproductive organs (local effects)	No effects on reproductive organs (local effects)	No effects on reproductive organs (mainly local effects)
Special studies on fertility	No data	No data	No data

Formaldehyde reacts at the site of entry and may not reach the reproductive organs. From morpholine the available information suggested no effects on reproductive organs. In summary, the available data on reproductive toxicity of MBM or the hydrolysis products give no indication for adverse effects on the reproductive organs.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information - MBM

Table 4.10-2 Developmental toxicity of MBM in rabbits

Route of exposure	Testtype Method Guideline	Species Strain Sex no/group	Exposure Period	Doses per day	Critical effects dams fetuses	NO(A)EL maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Reference
Oral Gavage	OECD guideline 414	Rabbit New Zealand White female 22	Gestation day 6-28	0, 10, 30, 100 mg/kg bw/ day in 1 ml corn oil/kg bw d	Local effects in the stomach No embryo- or fetotoxic properties	10 mg/kg bw/day or 1% in corn oil	100 mg/kg bw/day	Lubrizol (2005); MBM-Doc III A6.8.1

Summary and Conclusion (MBM-Doc III A6.8.1)

In a gavage study in rabbits (according to OECD guideline 414; see Table above; cf. MBM-Doc III A6.8.1) local effects (erosion and granular aspect of stomach) were found in the stomach of dams at ≥ 30 mg/kg bw/day corresponding to 3% in corn oil. Significant decrease in body weight gain and relative food consumption at 100 mg/kg bw/day corresponding to 10% in corn oil are considered to be secondary to these local effects. No developmental effects were detected at the high dose level of 100 mg/kg bw.

The implementation of a teratogenicity study in a 2nd species is scientifically unjustified because also no teratogenic effects are expected due to concentration dependent local effects.

4.10.2.2 Human information - MBM

No human data available.

4.10.2.3 Products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.8.1 & - Doc III A6.8.1)

Developmental toxicity studies performed to current test guidelines have not been available and an acceptable study in a non-rodent species has not been submitted. However, data in two rodent species (rat, mouse) do not indicate a teratogenic potential of formaldehyde after systemic exposure. Maternal toxicity, manifesting as body weight loss, was observed in rats following inhalation exposure to 47 µg/L x 6 h/d.

Embryofoetal toxicity was present at the same dose and resulted in decreased foetal weight and reduced or delayed ossification of thoracic vertebrae and sternal bodies. A gavage study in pregnant mice provided evidence of severe maternal and slight embryo-foetal toxicity at a dose of 185 mg/kg bw/d. No relevant effects on the dam or the foetus were observed at a dose of 148 mg/kg bw/d. Two further studies in dogs (Hurni and Ohder, 1973) and rats (Martin, 1990) were not considered suitable for risk assessment.

Overall, there is no concern for developmental toxicity of formaldehyde.

Morpholine (Morph-Doc II A4.8.1 & - Doc III A6.8.1)

Data on teratogenicity in rats after oral exposure to morpholine oleic acid salt (MOAS) are available (pregnant rats gavaged with 0, 234, 468, and 936 mg/kg bw/day on gestation day 6-15). The LOAEL for MOAS for maternal toxicity was 234 mg/kg bw/day (NOAEL < 234 mg/kg bw) corresponding to a LOAEL for morpholine of 88 mg/kg bw day; the described effects are not clearly systemically. No developmental effects were detected in any treatment group.

4.10.2.4 Comparison of MBM with products of hydrolysis

Table 3.8-2 Comparison of data on teratogenicity

Exposure route	MBM	Formaldehyde	Morpholine
Dermal exposure	No data but corrosive properties	No data but corrosive properties	No data but corrosive properties
Inhalation	No data local effects expected	maternal effects in rats NOAEC = 24 µg/L x 6 h/d developmental effects NOAEC = 24 µg/L x 6 h/d	No data local effects expected
Oral exposure	maternal effects in rabbits LOAEL 30 mg/kg bw/day (or 3% in corn oil) NOAEL10 mg/kg bw/day (or 1% in corn oil) developmental effects NOAEL 100 mg/kg bw/day (or 10% in corn oil) LOAEL>100 mg/kg bw/day	maternal effects in mice LOAEL = 185 mg/kg bw d NOAEL = 148 mg/kg bw d developmental effects LOAEL = 185 mg/kg bw d NOAEL = 148 mg/kg bw d	maternal effects in rats LOAEL 88 mg/kg bw/day NOAEL < 88 mg/kg bw/day developmental effects LOAEL > 353 mg/kg bw/day

MOAS: morpholine oleic acid salt

4.10.3 Summary and discussion of reproductive toxicity

No specific fertility study is available for MBM, but repeated dose toxicity data did not show treatment related histopathological effects in the reproductive organs. Local effects at the site of contact are to be expected. The hydrolysis product formaldehyde reacts at the site of entry and may not reach the reproductive organs. From morpholine the available information suggested no effects on reproductive organs. In summary, the available data on reproductive toxicity of MBM or the hydrolysis products give no indication for adverse effects on the reproductive organs.

In the oral developmental study in rabbits local effects in dams were found after exposure to MBM but no developmental toxicity even at the high dose level. After hydrolysis irritant/corrosive effects of the hydrolysis product formaldehyde are expected. For Formaldehyde local maternal effects but no primary developmental effects have been reported.

No systemic developmental effects have been reported for morpholine oleic acid salt in rats although maternal toxicity was evident.

In summary, developmental toxicity of MBM or the hydrolysis products formaldehyde and morpholine is only expected secondary to local maternal effects.

4.10.4 Comparison with criteria

The available data on potential adverse fertility effects or adverse developmental effects are conclusive and do not indicate evidence sufficient for classification.

4.10.5 Conclusions on classification and labelling

No classification for reproductive toxicity is necessary.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

No animal or human data were available on sexual function and fertility. There is, according to the DS, no evidence for such effects from the repeat dose studies.

There is one OECD TG 414 study in rabbits. No adverse effects on sexual function and fertility or development were seen. The DS proposed no classification.

Comments received during public consultation

One MSCA supported no classification for reproductive toxicity.

Assessment and comparison with the classification criteria

Fertility

Morpholine
According to the DS (and the applicant) no studies were available which are directly related to this endpoint (e.g. OECD TG 415, 416, or 422). In subchronic and chronic inhalation studies in rats the reproductive organs were investigated and no adverse effects were found. Consistent with this, in a long-term drinking water study in mice exposed to morpholine oleic acid salt no effects were also detected in the reproductive organs.

Formaldehyde
The Formaldehyde Core Document summarised repeated (14-day or 90-day) inhalation studies on rats which revealed testis atrophy, reduced sperm counts and motility and increased sperm abnormalities or reduced serum testosterone at doses which influenced food consumption and body weight gain. As no quantitative information on the reduction in food consumption and bw gain is reported, no conclusion can be drawn. Studies with intraperitoneal application confirmed adverse effects on sperm.

MBM
From the (14-day range-finding and) 90-day study on MBM there was no indication of effects on the reproductive organs in males and females.

There were no other specific studies on reproductive effects that assessed the sexual function and fertility effects of MBM.

In conclusion, no concern on fertility effects from the available repeated dose studies was identified. **RAC agrees that due to the lack of specific studies, no conclusion on effects on sexual function and fertility can be drawn and based on the currently available data classification for this endpoint is not warranted**.

Developmental toxicity

Morpholine
Data on teratogenicity in rats after oral exposure to morpholine oleic acid salt (MOAS) are available (pregnant rats gavaged with 0, 234, 468, and 936 mg/kg bw/d on gestation day 6-15). The LOAEL for MOAS for maternal toxicity was 234 mg/kg bw/d (NOAEL < 234 mg/kg bw/d) corresponding to a LOAEL for morpholine of 88 mg/kg bw/d. No developmental effects were detected in any treatment group. The information was taken

from the tables and English language abstract of a study published in Japanese (Sakemi et al., 2000, see doc. App. Morpholine).

Formaldehyde
No teratogenic effects were observed in inhalation or oral developmental toxicity studies conducted according to OECD TG 414. Fetotoxic effects (lower bw and retardations) were observed at the high dose with maternal toxicity (bw loss) (see Formaldehyde Core Document).

MBM
In a gavage study in rabbits (conducted according to OECD TG 414; cf. MBM-Doc III A6.8.1) local effects (erosion and granular aspect of stomach) were found in the stomach of dams at ≥ 30 mg/kg bw/d on GD 6-18 (3% MBM in corn oil). Significant decreases in body weight gain and relative food consumption at 100 mg/kg bw/d (10% MBM in corn oil) are considered to be secondary to these local effects. No developmental effects were detected at the high dose level of 100 mg/kg bw/d.
The RMS evaluation revealed that the gravid uterus weight was significantly reduced at 100 mg/kg bw/d. This seems to have been caused by a combination of (non significant) reductions of empty uterus weight, reductions of fetal weight and increases in resorptions. The latter two were also without a clear dose-response relationship. Delayed ossification at several sites, but without dose-dependency, was also reported.

The summary (MBM Doc III A6.8.1) stated that there were some premature deaths in all groups due to mis-dosing; these deaths were not test-substance related (no further details available from the summary). White spots and some haemorrhages in the stomach of all treated dams and controls found at necropsy were found to be most probably due to the stomach tube itself. Due to the limited study quality it is difficult to decide on the MBM-related effects in the gastrointestinal tract.

Testing in a 2nd species was considered as scientifically unjustified because also no teratogenic effects are expected due to concentration dependent local effects.

From the developmental study available (in rabbits) no adverse developmental effects were identified, therefore **no classification with regards to this endpoint is proposed by RAC**.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity- MBM

Summary and Conclusion (MBM - Doc III A6.4.1)

The subchronic study on rats according to OECD guideline 408 (cf. MBM-Doc III A6.4.1) included also functional observations at the end of exposure period including sensory reactivity to different types of stimuli (auditory, visual, proprioreceptive), assessment of grip strength and motor activity. Furthermore, detailed clinical observations were made once a week. No effects of toxicological relevance were reported.

4.11.1.2 Neurotoxicity – products of hydrolysis

Formaldehyde (Formaldehyde – Doc II A3.9 & - Doc III A6.9)

No evidence of neurotoxicity was reported in the repeated dose toxicity studies described in 3.5 and 3.7 of this document. However, studies conducted to assess specific behavioural consequences of formaldehyde inhalation in rats (Malek et al., 2003a and 2003b) measured an acute decrease of exploratory behaviour (open field locomotion, sniffing) and showed impairment of learning ability (increased error rate) in a water maze test conducted once per day over 10 days, 2 hours after the end of a daily 2-hour exposure to formaldehyde vapours. Learning was affected at the lowest dose tested of 0.12 µg/L (0.1 ppm) in males and females. However, exposure concentrations are considered not reliable and may have been approx. 1.2, 2.4 and 6 µg/L (1, 2 and 5 ppm) (Malek et al., 2004). In addition, ≥ 3.1 µg/L was required in a preceding 90-d rat

study to produce an equivalent effect (Pitten et al., 2000). Lu et al. (2008) reported that learning as assessed using the water maze was negatively affected and associated with oxidative stress in mice exposed to 3 µg/L formaldehyde gas for 6 h/d over 7 days, but not at the lower dose of 1 µg/L. Therefore, the effects observed are considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health remains unlikely.

Morpholine

No data specialized inhalation studies in experimental animals are available. There is no indication for neurotoxicity in repeated dose toxicity studies.

4.11.1.3 Neurotoxic effects of MBM compared with products of hydrolysis

Table 4.11-1 Comparison of data on neurotoxicity

	MBM	Formaldehyde	Morpholine
Effects	No neurotoxic effects in a subchronic gavage study in rats	effects observed are considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health remains unlikely	No indication for neurotoxicity in repeated dose toxicity studies

In conclusion, data available on MBM and morpholine did not suggest any neurotoxic properties.

4.11.1.4 Immunotoxicity

No Data available.

4.11.1.5 Specific investigations: other studies

No Data available.

4.11.2 Human information

No Data available.

4.11.3 Summary and discussion

Please see summary in 4.11.-1 above.

4.11.4 Comparison with criteria

No organ specific effects were observed in the studies with MBM and the hydrolysis products.

4.11.5 Conclusions on classification and labelling

No classification for STOT SE or RE is necessary.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Preliminary note: The references and results to key studies are highlighted bold throughout this chapter. For all key studies Robust Study Summaries are attached in Doc. III format.

Please note that Formaldehyde has been assessed by Germany as Rapporteur Member State for the Biocides Review Programme. For conclusions and results on the fate and behaviour in the environment and the environmental effects assessment of Formaldehyde reference is made to Appendix "Formaldehyde Core Dossier" (Version May 2012). For all Formaldehyde key studies Robust Study Summaries are attached in

Doc. III format. For Morpholine further information is attached in the Appendix "Morpholine" with Robust Study Summaries for key studies.

5.1 Degradation

5.1.1 Stability

5.1.1.1 Stability – MBM

Hydrolysis (MBM Doc. III-A 7.1.1.1.1/01 and /02)

The hydrolysis of N,N'-Methylenebismorpholine was studied according to a preliminary test proposed by Method C.7 of Commission Directive 92/69/EEC, which is not mentioned in OECD guideline 111. The principle of the preliminary test is that the hydrolysis half-life of a substance at 25°C can be expected to be less than 1 day, if the hydrolysis half-life is less than 2.4 hours at 50°C.

Samples at a concentration of 0.5 g/L were incubated in 4 buffered aqueous solutions for 2.4 hours at 37°C for pH 1.2, and at 50°C for pH 4, 7 and 9 for 2.4 hours. Aliquots of the sample solutions were analysed by gas chromatography.

Hydrolysis of N,N'-Methylenebismorpholione was very rapid, so that its concentration was below the limit of quantification of 3.18 mg/L at all times of measurement. The times of measurement within the test duration of 2.4 hours were not identified. Therefore, a more precise determination of the kinetic was not possible. Referring to the principle of the preliminary test, the DT_{50} was estimated to be less than 1 day at 25°C. As no MBM was detectable after 2.4 hours at 50°C, at 25°C, the DT_{50} it is also estimated to be significantly smaller than 1 day. Transformation products were qualitatively identified as Morpholine and an Aldehyde which was considered to be Formaldehyde.

Diluted to conceivable concentrations which may be expected at relevant environmental conditions (in waste waters or surface waters, excess amounts of water) as well as in human body fluids, N,N'-Methylenebismorpholine is expected to hydrolyse quickly and completely to Formaldehyde and Morpholine. Assuming MBM is applied as low-concentrated aqueous solution the transformation to Formaldehyde and Morpholine is expected to be almost total and already completed during the timeframe of storage and use (~hrs, days).

Table 5.1.1.1-1 Hydrolysis of N,N'-Methylenebismorpholine

Guideline / Test method	**pH**	**Temperature [°C]**	**Initial TS concentration, C_0 [mol/l]**	**Reaction rate constant, K_h [1/s x 10^5]**	**Half-life, DT_{50} [h]**	**Coefficient of correlation, r_2**	**Reference**
92/69/EEC C.7	1.2, 5, 7, 9	25°C	0.5 g/L	not applicable	**< 1 d**	not applicable	**MBM Doc. III-A 7.1.1.1.1/01 and /02,**

Conclusion:

MBM hydrolyses rapidly to Formaldehyde and Morpholine. Both products of hydrolysis are readily biodegradable (see section 5.1.2.2.2) and are like MBM not harmful or toxic to non-target aquatic organisms (see section 5.3). Therefore hydrolysis of MBM is considered as a proof for rapid degradation, fulfilling the Criteria given in the Guidance for the application on CLP criteria v. 3.0.

Photolysis in water **(MBM Doc. III-A 7.1.1.1.2)**

There is no study on photolysis of N,N'-Methylenebismorpholine in aqueous solution available. The UV spectrum indicates no absorption of light at wave-lengths >290 nm (cf. **MBM Doc. III-A 3.4**). The US EPA method OPPTS 835.2210 states that the test method is applicable to all chemicals which have an UV-absorption maximum in the range of 290-800 nm. Chemicals with UV absorption maximum of < 290 cannot undergo direct photolysis in sunlight. Therefore, the substance is not expected to be a candidate for noteworthy photolysis in sunlight and the performance of a test is concluded to be not necessary. Regarding this and the quick hydrolysis of MBM, phototransformation in water is not expected to be of relevance under environmentally relevant conditions.

Phototransformation in air **(MBM Doc. III-A 7.3.1)**

The UV spectrum shows no absorption of light at wave-lengths > 290 nm (cf. **MBM Doc III-A 3.4**). The US EPA method OPPTS 835.2310 states that the test method is applicable to all chemicals which have a UV absorption maximum in the range of 290-800 nm. Chemicals with UV absorption maximum of <290 nm cannot undergo direct photolysis in sunlight. Therefore, the substance is no candidate for any significant direct photolysis in sunlight.

The photochemical oxidative degradation of MBM was calculated using the computer simulation software AopWin v1.92. An overall OH rate constant of $3.62 \cdot 10^{-10}$ cm^3/molecule-sec was determined, resulting in an estimated half-life in air of 1.06 hours at 25°C (assuming $5x10^5$ OH/cm^3) **(cf. MBM Doc. III-A 7.3.1)**. Degradation by ozone is expected to be not relevant due to the absence of double bonds. Reaction with NO_3-radicals is estimated to be of minor relevance and to be covered by the reaction with OH-radicals.

Referring to the volatility of the substance, MBM reveals a vapour pressure of 0.625 Pa and a Henry's Law constant of $2.72x10^{-5}$ Pa·m^3/mol (cf. **Doc. III-A 3.2)**. Considering these low values, the quick degradation by OH-radicals in air and quick hydrolysis in the presence of water, gaseous release and accumulation in air are not considered to be relevant under environmentally relevant conditions.

Table 5.1.1.1-2 Phototransformation in air

Guideline / Test method	**Molecule / radical**	**Rate constant**	**Molecule/Radical concentration**	**Half-life (τ1/2)**	**Reference**
Estimation direct photolysis	h υ	0 (expected)			**MBM Doc. III A 7.1.1.1.2**
Estimation indirect photolysis (Calculation AopWin v1.91)	OH	$3.62 \cdot 10^{-10}$ cm^3/molecule s	$0.5 \cdot 10^6$ / cm^3 (24 h-day)	**1.06 h**	**MBM Doc. III A 7.3.1**
	Ozone	Negligible compared to reaction with OH radicals			
	NO_3	Negligible compared to reaction with OH radicals			

Conclusion:

The amount of MBM in the atmosphere is considered too low and its atmospheric lifetime is too short to have negative effects like stratospheric ozone depletion. Interaction of MBM with relevant atmospheric processes is expected to be negligible.

5.1.1.2 Stability - products of hydrolysis

Formaldehyde

Hydrolysis

Hydrolysis of Formaldehyde can be excluded because of the absence of a hydrolysable group in the molecule. However, at room temperature formaldehyde undergoes essentially complete hydration in water forming the formaldehyde hydrate "methylene glycol" ($CH_2(OH)_2$) and its oligomers, namely the low molecular mass poly(oxymethylene)glycols with the following structure $HO(CH_2O)nH$ (n = 8). For detailed information see **Formaldehyde Core Dossier**.

Photolysis in water

There are no tests on photolysis of Formaldehyde in aqueous solutions available which would allow deriving a reaction rate for surface waters. In aqueous solutions formaldehyde hydrate is formed which has no chromophore that is capable of absorbing sunlight and thus **should not decompose by direct photolysis.** Because of the ready biodegradability, photolysis in surface waters is expected to be of minor importance.

Photo-transformation in air

In the gas phase, Formaldehyde is rapidly degraded in air via reaction with OH radicals. The **half-life was estimated to be** 1.7 days and **1.97 in another calculation**. Degradation by nitrate and ozone is negligible. The decomposition by direct photolysis is 1.5 times higher than by OH radicals. The main transformation products are Hydrogen and Carbon monoxide.

Based on the half-life constants of formaldehyde, accumulation in the atmosphere is not to be expected. Furthermore, the Henry's law constant is relatively low. Therefore, Formaldehyde is not expected to volatilise to air from water surfaces in significant quantities and the amount which reaches the air compartment will be washed out by rain.

Conclusion:

The amount of Formaldehyde in the atmosphere is considered too low and its atmospheric lifetime is too short to have negative effects like stratospheric ozone depletion. Interaction of Formaldehyde with relevant atmospheric processes is expected to be negligible.

Morpholine (see Appendix Morpholine, Doc. III-A 7.1.1.1_morpholine and Doc. III-A 7.3.1_morpholine)

Hydrolysis

Under normal field conditions, it is assumed that **Morpholine is stable to hydrolysis**. However, no experimental data are available to confirm this.

Photolysis in water

As Morpholine shows no absorption in the UV spectrum (lambda > 260 nm), **direct photochemical degradation in the hydrosphere and in the atmosphere is unlikely**.

Photo-transformation in air

Morpholine will react with photochemically-produced hydroxyl radicals in the atmosphere. The **atmospheric half-life of Morpholine resulting from this reaction is estimated to be 2.6h**.

Conclusion:

The amount of Morpholine in the atmosphere is considered too low and its atmospheric lifetime is too short to have negative effects like stratospheric ozone depletion. Therefore nteraction of Morpholine with relevant atmospheric processes is expected to be negligible.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available

5.1.2.2 Screening tests

5.1.2.2.1 Screening tests - MBM

Ready Biodegradability

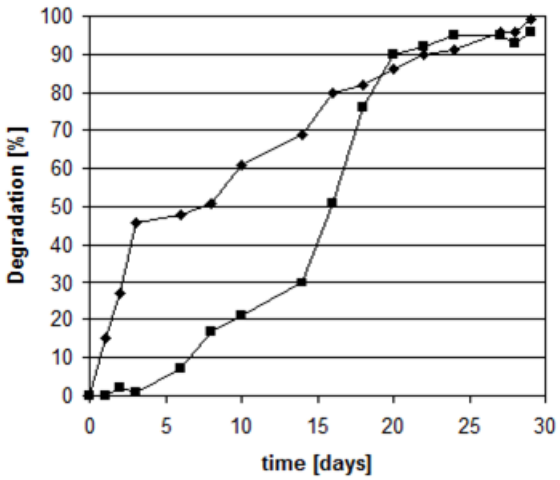
Table 5.1.2.2.1-1 Ready biodegradability (MBM and its hydrolysis products)

Guideline / test method	Test type	Test para-meter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Reference/ Reliability
			Type	Concen-tration	Adap-tation			Incubation period	Degree [%]	
OECD 301B ("CO2 Evolution") **GLP**	**ready**	**CO_2-Evolution**	**activated sludge**	**30 mg ss/L**	**no**	**no**	**17.2 mg a.s./L (10 mg C/L)**	**28 days**	**93% CO_2 evolution**	**MBM Doc III-A 7.1.1.2.1, Klimisch 1**

The biodegradability of N,N'-methylenebismorpholine was investigated in a study (cf. **MBM Doc. III-A 7.1.1.2.1, study A 7.1.1.2.1)** on ready biodegradability according to OECD Guideline 301B ("CO_2 Evolution Test"). The test results are summarized in Table 5.1.2.2.1-1. MBM is miscible with water in all proportions and hydrolyses rapidly in water to an aldehyde, which is likely Formaldehyde and Morpholine (DT_{50} <1 day) (cf. **MBM Doc. III-A 7.1.1.1.1/01 and /02**, ref. to section 5.1.1.1 Hydrolysis). Hydrolysis is expected to occur in all aquatic compartments and in wastewater. Therefore, the test results can be attributed to the parent compound as well as to the hydrolysis products.

Further study details: The inhibition to microorganisms was not specified in the study. However, a study according to OECD 209 resulted in a 3 h-NOEC of 32 mg/L (cf. MBM Doc III A7.4.1.4). As a reference substance sodium benzoate at a concentration of 17.1 mg/L was used. As an inoculum activated sewage sludge micro-organisms (source: aeration stage of a sewage treatment plant, predominantly domestic sewage) The sample was maintained on continuous aeration upon receipt. The inoculum for exposure was prepared by washing by settlement and resuspension in culture medium for three times to remove excessive amounts of dissolved organic carbon, no pretreatment. Initial cell concentration was 30 mg suspended solids/L. Sealed culture vessels with 3 L and 2 culture flasks/concentration were used. Aeration with CO_2-free air, rate of aeration: 40 mL/minute under continuous stirring, CO_2 and DOC were analyzed with a suitable equipment. No additional substrate was used, test temperature was 21 °C, pH 7.4, the dilution water was aerated overnight. For the preparation of the test solution 1000 mg of the test material was dissolved in culture medium followed by ultrasonification for 10 min and the volume adjusted to give 100 mL stock solution. An aliquot was dispersed in inoculated culture medium to give a final nominal concentration of 17.2 mg/L. Controls consisted of inoculated culture medium. Controls were performed in duplicate. Toxicity control consisted of test material (17.2 mg/L) plus reference substance (17.1 mg/L) in inoculated culture medium to give final concentration of 20 mg carbon/L (one vessel only). Calculation were performed according to OECD 301B.

Figure 5.1.2.2.1-1 Degradation curve (■st substance MBM, ◆eference)



An abiotic control was not performed. Degradation products were not monitored. Both pass levels (70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$ and the pass values reached within 10-d window (within 28-d test period) were fulfilled. The criteria for validity i.e. difference of extremes of replicate values of a.s. removal at plateau (at the end of test or end of 10-d window) <20% and the percentage of removal of reference substance reaches pass level by day 14 were both met. The study was assigned with reliability 1 according to the Klimisch cores (cf. Table 5.1.2.2.1-1).

Conclusion:
The OECD 301B test results show that MBM and its hydrolysis products are readily biodegradable (93% degradation based on CO_2 evolution within 28 days).

5.1.2.2.2 Screening tests - Products of hydrolysis
Formaldehyde

Ready Biodegradability

Table 5.1.2.2.2-1 Ready biodegradability of Formaldehyde

Method/ Guideline	Test type	Test para-meter	Inoculum			Add. sub-strate	Test substance Conc.	Degradation		Reference
			Type	Conc.	Adap-tation			Incubation period	Degree [%]	
OECD 301 D ("closed bottle test")	ready	BOD	not specified	no data	no data	no	2 - 5 mg form-aldehyde L^{-1}	28 days	90% of ThOD	Doc III-A7.1.1.2.1/01_HCHO Klimisch 3
OECD 301 C ("MITI-I test")	ready	BOD, TOC	activated sludge	sus-pended solids 30 mg L^{-1}	no data	no	100 mg paraform-aldehyde L^{-1}	14 days	91% of ThOD 97% of TOC	Doc III-A7.1.1.2.1/02_HCHO Klimisch 3
ISO 10707 ("closed bottle test")	ready	BOD	secondary effluent from laboratory municipal STP	0.5 ml L^{-1}	no	no	4 mg form-aldehyde L^{-1}	28 days	<60% of ThOD, (approx. 55%, visually deter-mined from the graph)	Doc III-A7.1.1.2.1/03_HCHO Klimisch 2
OECD 301A (" DOC Die-away test"	**ready**	**DOC**	**microorganisms from a digester of a sewage treatment plant with predominanty municipal wastewater**	**29.8 mg dry mass/L**	**no**	**no**	**10 mg DOC/L**	**28 days**	**99% of DOC, 10-d window fulfilled**	**Doc III-A7.1.1.2.1/04_HCHO Klimisch 1**

Formaldehyde was readily biodegradable in a test according to OECD 301 D ("closed bottle test", cf. Doc III-A7.1.1.2.1/01_HCHO). Depletion of dissolved oxygen was measured. The degree of degradation, expressed as percent of the theoretical oxygen demand (ThOD), amounted to 90% after 28 days. No information is available on the compliance with the 10-d window criterion. Because test performance is not reported in sufficient detail to evaluate the deviations from the international standard method including validity criteria, the study is only accepted as supportive information on the biodegradability of formaldehyde in a weight of evidence approach.
In a study according to OECD 301 C ("MITI-I test"), ready biodegradability of paraformaldehyde (polymer of formaldehyde, n = 8 - 100) was investigated (cf. Doc III-A7.1.1.2.1/02_HCHO). The degree of degradation, expressed as percent of the ThOD, amounted to 91% after 14 days. It is not reported if paraformaldehyde is completely dissolved in the study. Paraformaldehyde readily depolymerizes to formaldehyde solution by water e.g. in the presence of heat (Ullmann, 2005)[6]. Because test performance is not reported in sufficient detail to evaluate the deviations from the international standard method including validity criteria, the study is only accepted as supportive information on the biodegradability of formaldehyde in a weight of evidence approach.
Formaldehyde did not pass requirements for ready biodegradability in a closed bottle test according to ISO 10707 (cf. Formaldehyde Core Dossier, Doc III-A7.1.1.2.1/03_HCHO). The degree of biodegradation was approximately 55% of the ThOD after 28 days (visually determined from the graph). There is no information

[6] Ullmann (2005): Ullmann's Encyclopedia of Industrial Chemistry , 7th Edition, 2005

if all validity criteria are fulfilled in the study. In particular, the biodegradation of the reference substance is not reported. The study can be accepted but is not used as key study.

The key study of the Formaldehyde Core Dossier, **Doc. III-A 7.1.1.2/-04_HCHO** (reliability 1 according to the Klimisch Scores) tested biodegradation of Formaldehyde in a DOC Die-away test according to OECD guideline 301 A. The degree of DOC degradation was 99 % after 28 days. The 10-d window for Formaldehyde started on day 5 with the first value exceeding 10 % degradation. On day 5 the pass level of 70 % degradation has already been exceeded showing a DOC degradation of 91.9 %. Therefore, the criterion of the 10 d- window is fulfilled. The degradation of the reference substance sodium benzoate had reached 104 % within the first 14 days. The difference of extremes of replicate values of the removal of the test item at the end of the test and at the end of the 10-d window is less than 20 %. Therefore, the test can be considered as valid and is used as key study.
In addition, there are numerous other studies available, mainly from review articles and current publications. (cf. Doc. III-A 7.1.1.2_HCHO)

Conclusion:
On the basis of results from a study according to OECD 301A (cf. **Doc. III-A 7.1.1.2/-04_HCHO**) Formaldehyde is expected to be readily biodegradable.

Morpholine

Ready Biodegradability

Table 5.1.2.2.2-1 Ready biodegradability (Morpholine)

Guideline / test method	**Test type**	**Test para-meter**	**Inoculum**			**Additional substrate**	**Test substance concentr.**	**Degradation**		**Reference/ Reliability**
			Type	**Concen-tration**	**Adaptation**			**Incubation period**	**Degree [%]**	
OECD 301E ("Modified OECD screening test"), Non-GLP	ready	DOC removal	activated sludge from BASF treatment plant,	30 mg /L MLSS +	adapted	no	37 mg a.s./L	28 days	>90% DOC removal; lag period 15 days	Doc. III-A 7.1.1.2.1/01_morpholine, Klimisch 2
OECD 301E ("Modified OECD screening test"), Non-GLP	ready	DOC removal	activated sludge from laboratory-scale wastewater (municipal)	30 mg /L MLSS +	unadapted	no	37 mg a.s./L	28 days	**>90% DOC** removal; lag period 16 days	**Doc. III-A 7.1.1.2.1/01_morpholine, Klimisch 2**
No, not GLP	Sapromat test, using 3 different inocula	O2 consumption / substance dissapearance	3 different innocula (bacteria from river mud, wwtps, adapted bacteria)	unknown	only bacteria were adapted to amines as sole carbon source	Enriched by nutrients as in BOD test	10, 30, 100 mg/L	14 days	0%	Doc. III-A 7.1.1.2.1/02_morpholine, Klimisch 3
OECD 301F ("Manometric Respirometry Test")	ready	BOD	activated sludg with unclear sourcee	30 mg/L dry matter	unknown	no	100 mg a.s/L	28 days	>87% ThOD removal; lag period 16 days (sapromat) >89% ThOD removal; lag period 16 days (Oxitop)	Doc. III–A 7.1.1.2.1/03_morpholine,, Klimisch 2

+ MLSS mixed liquor suspended solids

A modified OECD screening test on ready biodegradability of Morpholine according to OECD 301E was performed using adapted and unadapted sludge. The test was conducted in an open system. Test results are summarized in Table 5.1.2.2.2-1. The DOC removal was greater than 90% using adapted and unadapted sludge. The degradation period was 5-7 days. No difference in the lag period of the unadapted (16 days) and adapted (15 days) sludge could be obtained. The degradation phase after passing the 10% level was below 10 days. Despite the limited information on test system and performance, adsorption or volatilization of Morpholine can be excluded, since no DOC removal occurred in the lag phase, the elimination in the abiotic control was less than 5% and the estimated K_{OC} for Morpholine (8 L/kg) suggests a very high mobility in soil (cf. **Doc. III–A 7.1.3_morpholine**, Doc. IV–A 7.1.3 HSDB, 2007). Therefore, Morpholine can be considered as readily biodegradable (cf. **Doc. III-A 7.1.1.2.1/01_morpholine, study A 7.1.1.2.1/01**).

Further study details (**Doc. III-A 7.1.1.2.1/01_morpholine)**: Test substance inhibitory to microorganisms: at 1000 mg/l inhibition of respiration activity (OECD 209) was 15%. Nitrification activity was not inhibited up

to 50 mg/L. Concerning materials and methods no information on test system, very limited information on test conditions, no information on pH, no information on TS preparation, water, stock solutions; mineral medium; preparation of flasks, number of flasks were provided. As a reference substance sodium benzoate (reported inhibition to microorganisms of 34.8 mg/L) was used.

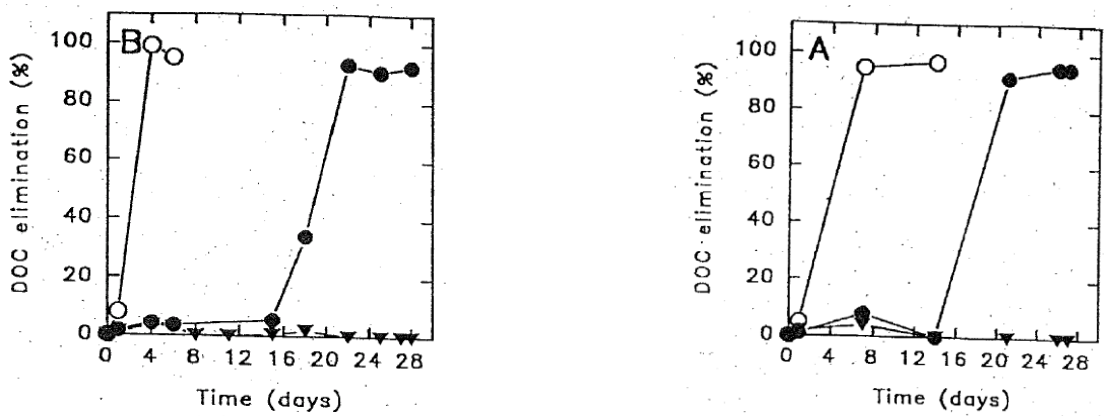
As an inoculum the test was conducted with 2 different inocula: 1. unadapted inoculum, supplied from a laboratory-scale wastewater treatment plant which was operated with municipal wastewater, 2. inoculum from the BASF treatment plant, Ludwigshafen (Germany), regarded to be adapted as Morpholine is regularly discharged to this treatment plant.

Information is not given on pretreatment of inoculums. Initial cell concentration was 30 mg MLSS[7]/L. No deviations from the test guideline were reported.

The test was conducted at 20°C on a rotary shaker. The initial test concentration was 37 mg/L, the duration of the test was 28 days. The analytical parameter was DOC removal with 7 -9 sampling sites. No intermediates or degradation products were identified. An abiotic control was reported (not inoculated, contamination by air prevented with 1 mL 1%(w/v) $HgCl_2$ per liter).

For the results please see Figure 5.1.2.2.2-1. The reference substance was degraded to almost 100% within ca. 5 days. Elimination in abiotic control was less than 5%.

Figure 5.1.2.2.2-1 Degradation curve, as given in the test report (o: sodium benzoate; ●: Morpholine, ▼: physical/ chemical elimination of Morpholine) of the 1: unadapted inoculum and 2: adapted inoculum



The pass levels of 70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$ as well as the pass values reached within 10-d window (within 28-d test period) were fulfilled. Concerning the criteria for validity no information was provided concerning the difference of extremes of replicate values of the test substance removal at plateau (at the end of test or end of 10-d window) < 20%. The criteria "percentage of removal of reference substance reaches pass level by day 14" was met. For reliability please see the Klimisch score listed in Table 5.1.2.2.2-1.

Another study investigated the biodegradability of amines. Morpholine was not degraded in 14 days in a sapromat test using river mud, sludge from wwtp and adapted bacteria (cf. study A 7.1.1.2/02_morpholine). The test was too short; no degradation can be expected within the given time frame. Micro-organisms capable of degrading Morpholine need already a minimum of 14 days to adapt and proliferate. Therefore, the study is only used supportively. In a later submitted study the biodegradability of Morpholine was tested according to OECD 301F (cf. Doc. III-A 7.1.1.2.1/03_morpholine, study A 7.1.1.2.1/01). Despite the fact that in the study report no information on adaptation is given, the study confirmed the long lag phase (16 days) and the readily biodegradability of the test substance.

Conclusion:

Morpholine is readily and therefore rapidly biodegradable.

[7] Mixed liquor suspended solids

Inherent Biodegradability

A Zahn-Wellens Test (OECD 302B) was performed to estimate the inherent biodegradability of Morpholine (cf. Doc. III-A 7.1.1.2.2_morpholine). The concentration of Morpholine was about 725 mg/L resulting in an initial DOC of 400 mg/L; test duration was 31 days instead of 28 days. Diethyleneglycole (845 mg/L) was used as a reference substance. The ratio of eliminated DOC could not be corrected for the blank, because no abiotic control had been conducted. The lag period for the adapted cultures in the ready test on Morpholine (modified OECD screening test, Table 5.1.2.2.2-1) was rather long, compared to the Zahn-Wellens test (7 days) carried out with the same inoculum. The shorter lag period might be due to the higher initial inoculum concentration in the inherent study (1 g/L MLSS) than in the ready test (30 mg/L MLSS). Results are given in Table 5.1.2.2.2-2. The sludge content in both test are according to the respective guideline. DOC removal was more than 90%.

Table 5.1.2.2.2–2 Inherent biodegradability (Morpholine)

Guideline / test method	Test type	Test para-meter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Reference/ Reliability
			Type	Concen-tration	Adaptation			Incubation period	Degree [%]	
OECD 302B ("Zahn-Wellens-Test")	inherent	DOC removal	activated sludge	1 g MLSS /L	Adapted	no	725 mg a.s./L (400 mg DOC/L)	31 days	> 90 % DOC removal; lag period 7 days	Doc. III-A 7.1.1.2.2_morpholine, Klimisch 2
OECD 302B ("Zahn-Wellens-Test")	inherent	DOC removal	activated sludge	1 g MLSS /L	Unadapted	no	725 mg a.s./L (400 mg DOC/L)	31 days	> 90 % DOC removal; lag period 20 days	Doc. III-A 7.1.1.2.2_morpholine, Klimisch 2

[+] MLSS mixed liquor suspended solids

Conclusion:

Since the substance exhibits a low potential for adsorption (see section 5.1.4.2) the results of the inherent tests show that Morpholine is ultimately inherently biodegradable and are therefore in line with the results of the ready tests.

5.1.2.2.3 Ready and inherent biodegradability – Summary and Conclusion

MBM

The OECD 301B test results (cf. **MBM Doc. III-A 7.1.1.2.1, study A 7.1.1.2.1**) show that MBM and its hydrolysis products are ready biodegradability (93% degradation based on CO_2 measurements within 28 days).

Formaldehyde

The ready biodegradability of Formaldehyde was investigated in 4 tests. Due to the results of a test according to OECD guideline 301A (cf. **Doc. III-A 7.1.1.2.1/04_HCHO**) Formaldehyde is readily biodegradable.

Morpholine

The OECD 301B test results (cf. **MBM Doc. III-A 7.1.1.2.1, study A 7.1.1.2.1)** showed that MBM and its hydrolysis products are ready biodegradability (93% degradation within 28 days). A modified OECD screening test on ready biodegradability of Morpholine according to OECD 301E using adapted and unadapted sludge (cf. **Doc. III-A 7.1.1.2.1/01_morpholine, study A 7.1.1.2.1/01**), showed also ready biodegradability. These results were confirmed by an OECD 301F test (cf. Doc. III-A 7.1.1.2.1/03_morpholine, study A 7.1.1.2.1/03). In addition a Zahn-Wellens Test (OECD 302B) was performed to estimate the inherent biodegradability of Morpholine (cf. Doc. III-A 7.1.1.2.2_morpholine, study A 7.1.1.2.2). DOC removal was >90% with both tests, irrespective if adapted or unadapted sludge was used.

5.1.2.3 Simulation tests –additional information

5.1.2.3.1 Simulation tests – Products of hydrolysis

Formaldehyde

Wastewater

In wwtp simulation tests comparable to OECD 303A (Confirmatory Test) Formaldehyde was removed by

99.5% and 99.4% under aerobic conditions (cf. Formaldehyde Doc. III-A 7.1.2.1.1/01 and /02_HCHO). The relatively high COD content in the effluent (18%) can be explained by disproportionation of Formaldehyde to Methanol and Formic acid. In the units, Ammonium was removed around 99.9%, indicating that there was no inhibition of nitrification. The hydraulic retention times (2.4 and 0.5 days) in the test unit is above the value (6 hours) proposed by the OECD Guideline 303A. Therefore, the resulting removal rates are assumed to probably overestimate removal in biological treatment plants. A test on anaerobic degradation (cf. Formaldehyde Doc. III–A 7.1.2.1.2_HCHO) reveals that Formaldehyde is rapidly removed in anaerobic digester sludge. All mentioned studies were accepted as supportive information. In conclusion, in the wwtp simulation tests, Formaldehyde was removed to 99.5% under aerobic conditions. The test on anaerobic degradation reveals that Formaldehyde is rapidly removed in anaerobic digester sludge (cf. **Formaldehyde Core Dossier**).

Morpholine

Wastewater

In several early studies all employing unadapted inocula and biological oxygen demand (BOD) as test parameter, Morpholine was found to be resistant to biodegradation (cf. Doc. III–A 7.1.1.2_morpholine, A 7.1.2 and A 7.2.1 Biodegradation additional Info).

But in a laboratory-scale wastewater treatment plant, which was operated with municipal wastewater and supplemented with 4.5 – 5.0 mg Morpholine/L, the TOC degradation ranged between 80 and 94% after an adaptation period of 10 to 12 days. Ammonia was quantitatively removed by nitrification. The concentration of the activated sewage sludge was between 0.6 and 2 g/L mixed liquid suspended solids (MLSS), the volumetric loading rate ranged between 0.1 and 0.68 kg TOC m^{-3} d^{-1} and the HRT was between 10 – 15h (cf. Doc. III–A 7.1.1.2, A 7.1.2 and A 7.2.1_morpholine Biodegradation additional Info, study Mor A 7.1.1.2.1/01).

In addition, the kinetics of the Morpholine degradation was determined within the study using a die-away test (cf. Doc. III–A 7.1.1.1.2, A 7.1.2 and A 7.2.1_morpholine Biodegradation additional Info, study A 7.1.1.2.1/01). After addition of 40 mg/L of Morpholine, 65% were degraded after 20 hours. After 25 hours less than 10% of the starting concentration was still present. The maximum degradation rate was 3 mg g $MLSS^{-1}h^{-1}$. Authors concluded that Morpholine concentrations of 5 mg/L can be degraded in adapted wastewater treatment plants, whereas shock loading (35 mg Morpholine/L) result in high Morpholine effluent concentrations, if the HRT is insufficient.

In a model activated sludge plant run with industrial waste water it was found that when Morpholine was absent from the influent, the ability of the activated sludge to degrade this compound was subsequently reduced (cf. Doc. III–A 7.1.1.1.2, A 7.1.2 and A 7.2.1_morpholine Biodegradation additional Info). This was shown by an increase in the lag period before Morpholine degradation could be detected in a die-away test with more than 40 days incubation, and was attributed to a decline in the specific population of Morpholine-degrading microorganisms. It was shown that in pure cultures of mycobateria the Morpholine degradative phenotype was genetically unstable.

In general, the growth rate of organisms belonging to the genus *Mycobaterium* capable of degrading Morpholine is very low; therefore the sludge retention time (SRT) needs to be long to ensure degradation of Morpholine (SRT <8 days, incomplete removal of Morpholine, SRT <3 days, no removal of Morpholine at all). Morpholine biodegradation usually features a lag-phase of >14 days. In addition, if the hydraulic retention time is not sufficient, high concentrations of Morpholine are not degraded (study Mor A 7.1.1.2.1/02). In conclusion, the long adaptation period of microorganisms to Morpholine would impair wastewater treatment in many plants (cf. Doc. III-A 7.1.3_morpholine, HSDB 2007), but adapted wastewater treatment plants might remove Morpholine, which need to be discharged regularly, without any lag phase.

Morpholine can be subject to chemical and biological nitrosation to N-Nitrosomorpholine (NMOR) (WHO, 1996). NMOR is classified as Carc. Cat 2 and should be regarded as if it were carcinogenic to humans (WHO, 1998). N-nitrosomorpholine (NMOR) can be formed by reaction of aqueous solutions of nitrite with Morpholine or by reaction of gaseous nitrogen oxides in aqueous solutions of Morpholine (WHO, 1996).

Freshwater

A study investigating Morpholine degraders in river water in UK found (cf. Doc. III–A 7.1.1.1.2, A 7.1.2 and A 7.2.1_morpholine Biodegradation additional Info), that the numbers of Morpholine degraders increased and the lag times decreased in a die-away test as the water passed downstream, which might indicate a cumulative effect. In 29 samples of river waters it was observed that only 3 (all from water classed as very clean) failed to reveal Morpholine biodegradation, although in several sites the numbers were near the limits of detection. Knapp & Whytell (cited in Doc. IV 7.1.1.2, A 7.1.2 and A 7.2.1 WHO, 1996) concluded that it is unlikely that Morpholine degradation occurred in rivers because of the long lag period (6 – 50 days). Guideline studies on the degradation in surface water (incl. sediment) are missing.

5.1.2.4 Mechanism of biodegradation of the hydrolysis products – additional information

Formaldehyde

The mechanism of Formaldehyde degradation in wastewater has been exemplarily shown for a strain of *Pseudomonas putida* isolated from industrial plant sludge (Adroer et al., 1990). The degradation of Formaldehyde is initiated by a dismutation reaction, yielding Formic acid and Methanol as products. Degradation of the products began after exhaustion of Formaldehyde in the medium.

However, there are several pathways and enzymes involved that allow microorganisms to detoxify and use Formaldehyde as energy source (Murdanoto et al., 1997, Vorholt, 2002). Amato et al. (2007) describe four fates of Formaldehyde being possible through microbial metabolic pathways: (a) its assimilation by the Serine and/or Ribulose monophosphate pathways (the latter involves a decarboxylation), (b) its reduction to Methanol, (c) its oxidation to Formic acid, and (d) its reaction with Methanol forming (cf. section Mechanism of biodegradation, **"Formaldehyde Core Dossier"**).

Morpholine

Morpholine seems to be degraded by a limited range of microorganisms. Species capable of degrading Morpholine belong to the genera *Mycobacterium* and *Arthrobacter*. The catabolic pathway for Morpholine was studied (cited in Additional Information, Doc. III-A 7.1.3 WHO 1996). When Mycobacterium strain MorG was grown with Morpholine as sole source of carbon and nitrogen. The results indicated that Morpholine is initially catabolized to 2-(2-aminoethoxy) acetate which can be oxidatively cleaved to give rise to glycolate and indirectly to ethanolamine.

5.1.3 Degradation – Comparison of MBM with products of hydrolysis

Table 5.1.3-1 Comparison of degradation data

Endpoint	**N,N'-Methylenebis-morpholine**	**Formaldehyde**	**Morpholine**
Hydrolysis	**Yes**	**No**	**No**
Phototransformation in water	**Not expected**	**Not expected**	**Not expected**
Phototransformation in air	**$T_{1/2}$ = 1.06 h**	**$T_{1/2}$ = ca. 41 h**	**$T_{1/2}$ = 2.6 h**
Biodegradation	**Readily biodegradable**	**Readily biodegradable**	**Readily biodegradable**

In aqueous solution **MBM** hydrolyses under formation of Formaldehyde and Morpholine. At concentrations being relevant for the environment, total and fast **hydrolysis** (~hrs) of MBM is expected. The hydrolysis products themselves are stable to hydrolysis. Hydrolysis of **Formaldehyde** can be excluded because of the absence of a hydrolysable group in the molecule. **Morpholine** is assumed to be stable to hydrolysis under normal field conditions. However, no experimental data are available to confirm this.

However, both products of hydrolysis are readily biodegradable (see below) and are like MBM not harmful or toxic to non-target aquatic organisms (see section 5.3). Therefore hydrolysis of MBM is considered as a

proof for rapid degradation, fulfilling the Criteria given in the Guidance for the application on CLP criteria v. 3.0.

Phototransformation in the water phase is neither expected for **MBM** nor for its hydrolysis products **Formaldehyde and Morpholine**, because of absence of a chromophore. Additionally the quick hydrolysis of MBM and the ready biodegradation of MBM and its hydrolysis products, contribute to the fact that photolysis in surface waters is expected to be negligible.

The low vapour pressure and the low Henry's law constants indicate that both N,N'-methylenebismorpholine and its hydrolysis products are practically not volatile from aqueous solution. Thus, gaseous release and accumulation in air are not considered to be relevant under environmental conditions. If MBM or its hydrolysis products reach the atmosphere **rapid degradation through reaction with hydroxyl radicals** can be assumed.

Therefore the amount of MBM and its hydrolysis products in the atmosphere are considered too low and their atmospheric lifetimes are too short to have negative effects like stratospheric ozone depletion. Interaction of MBM and its hydrolysis products with relevant atmospheric processes is expected to be negligible.

Based on results of the N,N'-methylenebismorpholine biodegradation study the substance as well as its hydrolysis products are considered to be **readily biodegradable**. In addition the hydrolysis products Formaldehd and Morpholine have proven to be readily biodegradable in separate ready tests.

Conclusion:

N,N'-methylenebismorpholine and its hydrolysis products Formaldehyde and Morpholine are rapidly biodegradable. Furthermore it is not expected that MBM and its hydrolysis products have negative effects on the atmosphere like stratospheric ozone depletion.

5.1.4 Adsorption/Desorption

5.1.4.1 Adsorption/Desorption – MBM (cf. MBM Doc. III-A 7.1.3)

The adsorption coefficient for N,N'-methylenebismorpholine was determined by the HPLC method following the Draft OECD Guideline 121. The **K_{oc} value** was estimated to be **<17.8 L/kg.** The test was conducted at a neutral pH where the test substance is ionized. The unionized form is expected at pH >10.5, however this pH is not relevant for the environment (cf. MBM Doc. III-A 7.1.3).

The experimental value of <17.8 L/kg is supported by the calculated value of 10 L/kg using EpiWin v3.12 (cf. MBM Doc. III-A 7.1.3, Doc. IV-A 3.2).

5.1.4.2 Adsorption/Desorption – Products of hydrolysis

Formaldehyde

There is no study available on adsorption of Formaldehyde in soils and sediments. Therefore, the K_{OC} was estimated according a QSAR model described in EU Technical Guidance Document on Risk Assessment (EC 2003). Based on a log K_{OW} of 0.35 and the QSAR for non-hydrophobics, the **K_{OC} is** calculated to be **15.9 L/kg**. Therefore, Formaldehyde is expected to exhibit only a very weak adsorption in soils and sediments.

The HPLC-screening test according to OECD Test Guideline (TG) 121 is not feasible as it is outside the scope of the method. A request for a test according to OECD TG 106 will not improve the information on the distribution behaviour of Formaldehyde in terms of overall mobility. A current literature research revealed that no information is available on the adsorption behaviour of low-molecular aldehydes. For detailed information see **Appendix Formaldehyde Core Dossier**.

Morpholine (Doc. III-A 7.1.3_morpholine **Adsorption - Additional information)**

Using a measured log octanol/water partition coefficient (log K_{OW}) of -0.86 and a regression equation, the estimated **K_{OC} for Morpholine is 8 L/kg**. The K_{OC} for Morpholine estimated from molecular structure is 5 L/kg. These estimated K_{OC} suggest that Morpholine has a very high mobility in soil.

5.1.4.3 Adsorption/Desorption –Comparison of MBM with products of hydrolysis

Table 5.1.4.3-1 Comparison of distribution data

Endpoint	**N,N'-Methylenebismorpholine**	**Formaldehyde**	**Morpholine**
Distribution	**K_{oc} <17.8 L/kg (experimental)**	**K_{oc} = 15.9 L/kg (estimated)**	**K_{oc} = 8 L/kg (estimated)**

On the basis of their low K_{oc} values MBM and its hydrolysis products are expected to be highly mobile in soil.

5.1.5 Volatilisation

Table 515-1: Vapour pressure

Property	**Purity/Specification**	**Results**	**Reference**
Vapour pressure	100%	2.17 x 10^{-4} Pa (25°C) 2.096 x 10^{-4} Pa (20°C)	Doc. III-A 3; Study A3/01D
Henry´s Law Constant	n.a.	0.472 Pa x m^3 x mol^{-1} (calculated according to HENRYWIN 3.10)	Doc. III-A 3; Study A3/04

The transfer of a substance from the aqueous phase to the gas phase is estimated by means of its Henry´s Law constant.

K air-water = (HENRY) / (R*Temp) = 1.9*10^{-4}
With HENRY [Pa * m^3 *mol^{-1}], R = 8.314 Pa * m^3 –mol^{-1}*K^{-1}; Temp [K]

5.1.6 Distribution modelling

No data available.

5.2 Aquatic Bioaccumulation

5.2.1 Aquatic Bioaccumulation – MBM

5.2.1.1 Bioaccumulation estimation – MBM (cf. MBM Doc. III-A 7.4.2)

According to TGD (EC 2003, part II, chapter 3, p. 126) a BCF_{fish} for substances with a log K_{ow} of 2 - 6 can be calculated using the QSAR developed by Veith et al. (1979). However, the log K_{ow} value for N,N'-methylenebismorpholine was determined to be –1.53 and is outside of the domain of the QSAR. As a worst case approach, for a log K_{OW} of 1, a BCF_{fish} of 1.41 L/kg is calculated using the same QSAR equation (cf. **MBM Doc. III-A 7.4.2 bioconcentration aquatic - Justification for non-submission**).

Table 5.2.1.1-1 Estimations of aquatic bioconcentration of MBM

Basis for estimation	**log K_{OW} (calculated)**	**Estimated BCF for fish (freshwater)**	**Estimated BCF for fish eating bird/predator**	**Reference**
QSAR	-1.53	**1.41 L/kg**		**MBM Doc. III-A 7.4.2 – Justification for non-submission**

5.2.1.2 Measured bioaccumulation data – MBM

No experimental data on bioaccumulation are available. Due to the rapid hydrolysis of N,N'-methylene-bismorpholine (cf. **MBM Doc. III-A 7.1.1.1.1/01 and /02**), an experimental determination of the BCF is not possible.

5.2.2 Aquatic Bioaccumulation – Products of hydrolysis

5.2.2.1 Bioaccumulation estimation – Products of hydrolysis

Formaldehyd

In experimental studies on bioaccumulation no elevated Formaldehyde levels were found. Additional information on **log K_{OW} (0.35)** as well as the estimated **BCF_{fish} (0.396 L/kg)** and biomagnification factor for fish-eating predators (1) support the experimental findings that Formaldehyde does not accumulate in aquatic biota. For detailed information see **Appendix Formaldehyde Core Dossier**.

5.2.2.2 Measured bioaccumulation data – Products of hydrolysis

Morpholine (Doc. III-A 7.4.2_morpholine)

Morpholine is miscible with water and has a very low measured octanol/water partition coefficient (**log K_{OW} -0.86**). Consequently, its potential for bioconcentration in aquatic organisms is expected to be extremely low. An experimentally determined **BCF_{fish} for Morpholine was < 2.8 L/kg** (cf. **Doc. III-A 7.4.2**_morpholine **Additional Information**). In addition, the log K_{OA} (octanol/air) estimate is 3.5 (EpiSuite). The value is based on measured Henry Constant and log K_{OW}.

5.2.3 Aquatic Bioaccumulation – Comparison of MBM with products of hydrolysis

Table 5.2.3-1 Comparison of BCF values

Endpoint	**N,N'-Methylenebismorpholine**	**Formaldehyde**	**Morpholine**
Aquatic bioconcentration	**1.41 L/kg (calculated)**	**0.396 L/kg (calculated)**	**<2.8 L/kg (experimental)**

Overall, no significant bioaccumulation potential is expected for N,N'-methylenebismorpholine as well as for its hydrolysis products.

5.3 Aquatic toxicity

Classification is based on the key studies (results and references highlighted bold). For all key studies Robust Study Summaries are attached in Doc. III format.

N,N'-Methylenebismorpholine (abbreviation: MBM) hydrolyses rapidly in concentrations relevant for wastewaters and surface waters. In the media of the toxicity tests, the hydrolysis products Formaldehyde and Morpholine are present (cf. **Stability**); therefore the observed effects are caused by a mixture of the two

hydrolysis products. According to OECD No 23[8] Guidance document it is recommended for fast degrading substances (<3 days – 1 hour) to decide on a case-by-case basis weather the parent compound or the metabolites (in this case the hydrolysis products) should be tested.

5.3.1 Aquatic toxicity – MBM

The acute toxicity of N,N'-methylenebismorpholine to aquatic organisms was tested in several studies covering all three trophic levels (fish, daphnia, and algae). All acute tests were performed according to the respective OECD Guidelines No 203 (fish), 202 (daphnids) and 201 (algae).

The test solutions were prepared some hours before the test organisms were introduced ensuring complete hydrolysis of the test substance OS 157340 (=MBM, purity 98% w/w). In all acute tests the hydrolysis product Morpholine was monitored, in contrast to Formaldehyde, which was not analysed. The test organisms were therefore exclusively exposed to the hydrolysis products, instead of the parent substance. Monitoring revealed that the test substance (measured as Morpholine) was stable over the test period. The nominally confirmed concentrations based on Morpholine were used to derive the effect values for the test substance.

5.3.1.1 Fish - MBM

Short-term toxicity to fish (cf. MBM Doc. III-A7.4.1.1)

In a study on the acute toxicity of the hydrolysis products of N,N'-Methylenebismorpholine towards fish (cf. **MBM Doc. III-A7.4.1.1**) the LC_{50} was determined to be >100 mg/L (based on nominally confirmed concentration of Morpholine), employing the rainbow trout (*Oncorhynchus mykiss*) as test organism. No mortalities and no sub-lethal effects were observed in this study conducted with the hydrolysis products of MBM. Approximately 107 mg MBM/L (calculated) are necessary to generate 100 mg Morpholine/L and 18 mg Formaldehyde/L (16.7% w/w Formaldehyde release cf. **MBM Doc. III-A 4.1/02**). Morpholine was shown to be stable in the test medium for the duration of the test by HPLC analysis; the aldehyde was not analyzed in any of the samples.

Table 5.3.1.1-1 Acute toxicity of the hydrolysis products of N,N'-methylenebismorpholine to fish

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference/ Reliability
			design	duration	LC_0	LC_{50}	LC_{100}		
OECD 203	*Oncorhynchus mykiss*	Mortality	semi static	96 h	100 mg/L (n.c.)*	**>100 mg/L (n.c.) Morpholine ≅ 107 mg/L MBM (calc.) ≅ 18 mg/L Formaldehyde (calc.)**	-	Only one conc. tested, GLP, freshwater;	**MBM Doc. III-A7.4.1.1, Klimisch 1**

*n.c.: nominally confirmed concentration of the hydrolysis product Morpholine

Long-term toxicity to fish (cf. MBM Doc. III-A 7.4.3.2 – Justification for non-submission)

A study on chronic fish toxicity with N,N'-methylenebismorpholine as test substance is not available **(cf. MBM Doc. III-A 7.4.3.2 – Justification for non-submission)**. However, the available data on acute toxicity indicate that fish is the trophic level exhibiting the lowest sensitivity, as the LC_{50} (>107 mg/L, *Oncorhynchus mykiss*, cf. **MBM Doc. III-A 7.4.1.1, study A 7.4.1.1**) is much higher than the E_rC_{50} (10 mg/L) of the algae (*Pseudokirchneriella subcapitata*, cf. **MBM Doc. III-A 7.4.1.3, study A 7.4.1.3**). In addition, the comparison of aquatic toxicity data for N,N'-methylenebismorpholine and its hydrolysis products reveals that the toxicity of N,N'-methylenebismorpholine is primarily determined by its

[8] Guidance document on aquatic toxicity testing of difficult substances and mixtures: http://www.oecd.org/LongAbstract/0,2546,en_2649_34377_1915817_1_1_1_37407,00.html

Formaldehyde content (cf. chapter 4.2.1.4 Comparison of aquatic acute and chronic toxicity data of this document).

For Formaldehyde, an embryo-larval test on *Danio rerio* resulted in a 6 days LC_{50} of 6.9 mg/L (cf. **Formaldehyde Core Dossier Doc III A 7.4.3.2;** only supporting information; test duration should have been 8-10 days), while a test on acute toxicity with the same species resulted in a 96h LC_{50} of 41 mg/L (cf. **Formaldehyde Core Dossier Doc III A7.4.1.1/04**). The results reveal that the chronic toxicity does not increase substantially, when compared to the acute toxicity. Based on these findings, it can be assumed that N,N'-methylenebismorpholine does not cause specific negative effects on fish reproduction. Overall, it is unlikely that another chronic NOEC for MBM from fish would be lower than the NOEC available for the most sensitive taxonomic group - algae. Furthermore, testing of such species would be ethically questionable and contrary to animal welfare concerns.

Therefore, a test on chronic fish toxicity is scientifically not justified.

5.3.1.2 Aquatic invertebrates - MBM

Short-term toxicity to aquatic invertebrates (cf. MBM Doc. III-A 7.4.1.2)

The acute toxicity of the hydrolysis products of the test material OS 157340 (=MBM purity 98% w/w) to the freshwater invertebrate *Daphnia magna* has been investigated and gave a 48h EC_{50} value of 24 mg/L (based on nominally confirmed concentrations of the hydrolysis product, Morpholine) with 95% confidence interval of 20-30 mg/L (cf. **MBM Doc. III-A 7.4.1.2**). Approximately 26 mg MBM/L (calculated) were necessary to generate 24 mg Morpholine/L and 4.3 mg Formaldehyde/L (calculated). The NOEC based on zero immobilisation at 48 hours was 5.6 mg/L (based on the nominal concentration of the hydrolysis product, Morpholine). The 48h-EC_{50} value of 24 mg Morpholine/L (≅ 4.3 mg Formaldehyde/L) is in accordance with the lowest reliable 48h-EC_{50} of 5.8 mg/L found with Formaldehyde (cf. **Formaldehyde Core Dossier Doc. III A7.4.1.2/03**).

Table 5.3.1.2-1 Acute toxicity of the hydrolysis products of N,N'-methylenebismorpholine to invertebrates

Guideline/ Test method	Species	Endpoin/ Type of test	Exposure		Results			Remarks	Reference/ Reliability
			design	duration	EC_0	EC_{50}	EC_{100}		
OECD 202	*Daphnia magna*	Mobility	static	24 h	32 mg/L (n.c.)	71 mg/L (n.c.) 95% C.I. 61-83	n.d.	GLP, freshwater conc. ≥ 80% of nominal (via Morpholine)	MBM Doc. III A 7.4.1.2, Klimisch 1
				48 h	5.6 mg/L (n.c.)	**24 mg/L (n.c.)** 95% C.I. 20-30 mg/L **Morpholine ≅ 26 mg/L** **MBM (calc.) ≅ 4.3 mg/L Formaldehyde (calc.)**	100 mg/L (n.c.)	GLP, freshwater conc. ≥ 80% of nominal (via Morpholine)	**MBM Doc. III-A 7.4.1.2, Klimisch 1**

n.c.: nominally confirmed concentration of the hydrolysis product Morpholine, n.d. not determined

Long-term toxicity to aquatic invertebrates (cf. **MBM Doc. III-A 7.4.3.4**)

The effects of Contram^{TM} ST-1 (= MBM, purity >92%) on the reproductive output of *Daphnia magna* was tested according to the OECD guideline 211 (cf. **MBM Doc. III-A 7.4.3.4**). The test was performed under semi-static conditions using six different nominal test concentrations of "ST-1" (0.625, 1.25, 2.5, 5.0, 10.0,

and 20.0 mg/L) with 12 replicates per concentration and control. MBM was introduced as a stock solution prepared in an appropriate medium. Any evaporation of volatile components was avoided, by completely filling and by using glass stoppers. In the chronic study total Formaldehyde content (bound and free) was measured. Recoveries of spikes on basis of the nominal concentrations were in the range of 82.5 – 102.9%. Quantification at t=0 and after 24 hours of incubation revealed evidence that the test item remained stable at >80% of the initial concentration.

The dose-response relationship of the % inhibition of cumulative offspring of survivors by the test item after 21-days is unclear. The effect values at 2.5 and 10 mg/L ("ST-1" based on nominal concentration) were nearly identical with 18.9 and 19.7% offspring reduction. At 5 mg/L (n.c. of "ST-1") the effect was 9.2% offspring reduction and therefore lower than at 2.5 and 10 mg/L. It seems, that the mean reproductive output at the concentration of 2.5 mg/L is an outliner, thus 3 out of 10 data points (cumulative offspring of survivors) are much lower than the rest.

Table 5.3.1.2-2 Chronic toxicity of the hydrolysis products of "ST-1" (= MBM purity >92%) to invertebrates

Guideline/ Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**			**Remarks**	**Reference/ Relaibility**
			design	**duration**	**NOEC EC10 LOEC**	**EC_{50}**	**EC_{100}**		
OECD 211	*Daphnia magna* STRAUS *(clone 5);*	Cumulative offspring of survivors	semi-static	21 days	**NOEC 5 mg/L;** LOEC 10 mg/L; EC_{10} 6.4 mg/L. **MBM ≅ 4.7 mg Morpholine ≅ 0.8 mg Formaldehyde**	16.4 mg/L (n.c.)	-	GLP, freshwater, conc. total Formaldehyde (bound and free);	**MBM Doc. III-A 7.4.3.4 Klimisch 2**
OECD 211	*Daphnia magna* STRAUS *(clone 5);*	Mean offspring of survivors	semi-static	21 days	NOEC 10 mg/L; LOEC 20 mg/L; EC_{10} 4.4 mg/L. based on n.c.	20.5 mg/L (n.c)	-	GLP, freshwater, conc. total Formaldehyde (bound and free);	MBM Doc. III-A 7.4.3.4 Klimisch 2

n.c.: nominally confirmed concentration of MBM

5.3.1.3 Algae and aquatic plants – MBM

Short- and long-term toxicity to algae (cf. **MBM Doc. III-A 7.4.1.3)**

The effects of the hydrolysis products of the test material OS 157340 (= MBM, purity 98%) on the growth of algae (cf. **MBM Doc. III-A 7.4.1.3**) were investigated in the freshwater species *Pseudokirchneriella subcapitata*. The hydrolysis products were tested at the following nominal concentrations of Morpholine: 2, 4, 8, 16, and 32 mg/L. Morpholine was shown to be stable in the test medium for the duration of the test; the aldehyde was not analyzed in any of the samples. The test gave an E_bC_{50} (72h) value of 4.4 mg/L, an E_bC_{50} (96h) value of 4.2 mg/L and an E_rC_{50} (0-96h) value of 9.5 mg Morpholine/L (≅10 mg/L MBM and ≅1.6 mg Formaldehyde/L). The 72h NOE_rC was 2 mg/L, corresponding to ≅2.1 mg/L MBM and ≅0.36 mg/L Formaldehyde. All results are based on the nominally confirmed concentration of Morpholine. A re-growth study (216h) was conducted, which indicated the test material to be algistatic in effect.

Table 5.3.1.3-1 Growth inhibition of the hydrolysis products of N,N'-methylenebismorpholine to algae

Guideline/ Test method	**Species**	**Endpoin/ Type of test**	**Exposure**		**Results**			**Remarks**	**Reference/ Reliability**
			design	**duration**	**NOE_rC**	**E_bC_{50}[1]**	**E_rC_{50}[2]**		
OECD 201	*Pseudokirchneriella subcapitata* *CCAP 278/4*	Growth rate	static	96 h	**2.0 mg/L (n.c.) 72 h Morpholine) ≅ 2.1 mg MBM (calc.) ≅ 0.36 mg Formaldehyde (calc.)**	4.2 mg/L (n.c.)	**9.5 mg/L (n.c.)[3] Morpholine ≅ 10 mg/L MBM (calc.) ≅ 1.6 mg/L Formaldehyde (calc.)**	GLP, freshwater conc. ≥ 80% of nominal (via Morpholine)	**MBM Doc. III-A 7.4.1.3, Klimisch 1**

[1] calculated from the area under the growth curve; [2] calculated from growth rate; [3] n.c.: nominally confirmed concentration of the hydrolysis product Morpholine

Further study details:

Deviation from the guideline: Due to the rapid hydrolysis of the test material a modification of the standard method was performed. Toxicity of the hydrolysis product of N,N-methylenebismorpholine was studied after ensuring complete hydrolysis of the parent compound.

During the present study, no N,N-methylenebismorpholine was found after 3 hours. Morpholine was analysed with HPLC and UV/VIS. Linearity of the detection system was confirmed at 0 – 200 mg/L. The culture medium was prepared using reverse osmosis purified water (Elga Optoma 15+) and the pH was adjusted to 7.5± 0.1 with 0.1 N NaOH or HCl. The prepared media was sterilised by 0.2 µm membrane filtration and stored in darkness. The test organisms came from the Institute of Freshwater Ecology, Cumbria. The Initial cell concentration was 10^4 cells /mL.

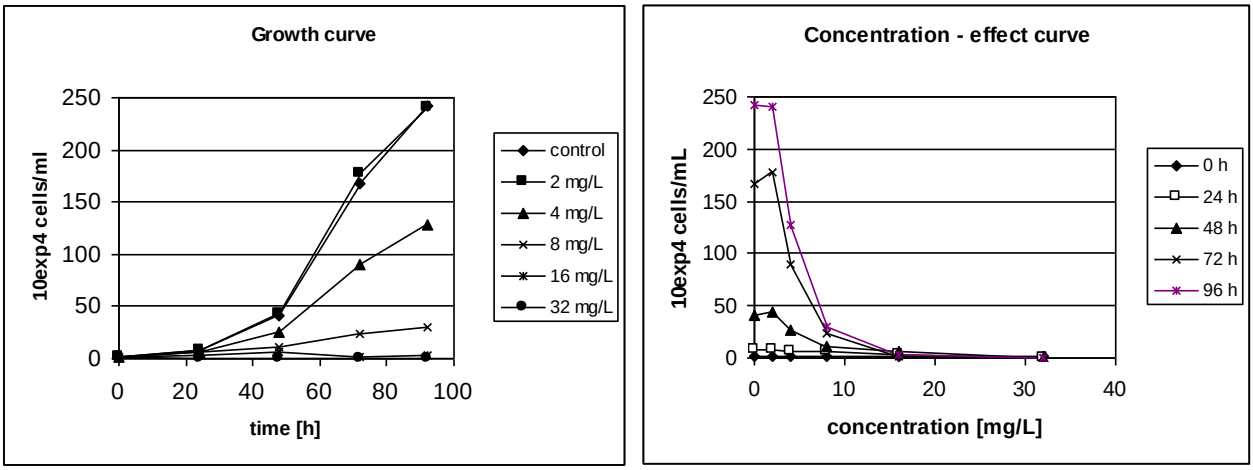
The test conditions were: test temperature: 24 ± 1°C, recorded hourly; pH: at start: 7.5 – 8.7 and at end of test: 7.4 – 10.1; aeration of dilution water: no; light intensity: 7000 lux; photoperiod: continuous illumination.

The test parameter was cell multiplication inhibition with daily sampling. Monitoring of the test substance took place at 0 and 96 hours. Statistics: One way analysis of variance incorporating Bartlett's test for homogeneity of variance Dunnett's multiple comparison procedure for comparing several treatments with a control SAS computer software package, 95% confidence limits are determined using the method of Litchfield and Wilcoxon (1949).

A range finding study was performed prior to the main test with 1, 10 and 100 mg/L (nominal concentrations). No effect was observed at 1.0 mg/L. Growth was observed to be reduced at 10 and 100 mg/L. In addition a slight precipitate was observed at 100 mg/L.

The results of the main test are shown in Figure 5.3.1.3-1. The analytical measurements reveal that the morpholine concentration was stable during the test period. Therefore results are presented as nominal concentrations.

Figure 5.3.1.3-1 Growth and concentrations-effect curve



The validity criteria "Cell concentration in control cultures increased at least by a factor of 16 within 3 days" and "Concentration of test substance ≥80% of initial concentration during test" were fulfilled. However the validity criteria according to OECD concerning the the mean coefficient of variation for section-by section specific growth rates in the control must not exceed 35% and the coefficient of variation of average specific growth rates during the whole test period in replicate controls must not exceed 7% in the tests with *P. subcapitata* are missing in the study. The pH increased more than 1.5 units.

5.3.1.4 Summary and Conclusion

In a study on the acute toxicity of the hydrolysis products of N,N'-Methylenebismorpholine to fish the LC_{50} (96h) for MBM was determined with >107 mg/L, (based on the nominally confirmed concentration of the hydrolysis product, Morpholine and employing rainbow trout (*Oncorhynchus mykiss*) as test organism. The test was designed as a limit test; the substance was only applied at 100 mg/L. No effects were observed at this concentration. A justification for non-submission of data was accepted for the chronic fish test, since fish was the least sensitive species in the acute toxicity studies.

Acute toxicity towards invertebrates was tested with the water flea *Daphnia magna*, and the EC_{50} (48h) MBM was established at 26 mg/L (based on the nominally confirmed concentration of the hydrolysis

product, Morpholine). In a chronic toxicity test against invertebrates *Daphnia magna* exhibited a NOEC of 5 mg/L (cumulative offspring).

Algal toxicity was found to be higher with an E_rC_{50} (72h) for MBM of 10 mg/L. The NOE_rC (72h) for MBM based on the growth rate was determined to be 2.1 mg/L. Re-growth experiments (216h) revealed that the hydrolysis products had an algistatic effect.

Acute and chronic aquatic studies demonstrated that algae is the most sensitive species showing the lowest short- and long-term effect values after exposure to the hydrolysis products of N,N'-methylenebis-morpholine.

5.3.2 Aquatic toxicity – Products of hydrolysis

5.3.2.1 Aquatic toxicity - Formaldehyde

Acute and chronic toxicity tests covering all 3 trophic levels are available for Formaldehyde. All studies were conducted without analytical monitoring. However, due to the very low Henry's law constant and the low adsorption potential of Formaldehyde in water, losses of test substance due to volatilisation or adsorption during exposure are assumed to be insignificant. Therefore, results based on the nominal concentrations can be regarded as valid. For detailed information cf. **Appendix Formaldehyde Core Dossier**.

Short-term toxicity to fish (cf. **Formaledhyde Doc. III-A 7.4.1.1/05**)

LC_{50} values for fish for Formaldehyde are in the range of 1.8 mg Formaldehyde/L (*Morone saxatilis* cf. **Formaledhyde Doc. III-A 7.4.1.1/05_HCHO**) to 69 mg Formaldehyde/L (*Oncorhynchus mykiss* Formaledhyde Doc. III-A 7.4.1.1/03).

The lowest reliable effect value of **5.7 mg/L** was obtained with the species *Morone saxatilis* (cf. Formaldehyde **Doc. III A7.4.1.1/05_HCHO**). This study was performed in freshwater with fish acclimated to these conditions, although *Morone saxatilis* is a marine species typically living in coastal waters and bays, but also enters the rivers.

Table 5.3.2.1-1 Acute toxicity of Formaldehyde to fish (ref. to Formaldehyde Core Dossier Table 4-6)

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**			**Remarks**	**Reference**
			design	**duration**	**LC_0**	**LC_{50}**	**LC_{100}**		
National guideline (Germany), Vom Wasser 46, 291-295 (1976)	*Leuciscus idus* (golden orfe)	mortality	static	48 hours	9.6 mg/L (n.c.)	15 mg/L (n.c.)	22.8 mg/L (n.c.)	related to Form-aldehyde; results from tests performed in 2 indipendent laboratories ; no GLP	FA Doc. III A 7.4.1.1/01_ HCHO Klimisch 3
Non guideline study	*Morone saxatilis* (striped bass)	mortality	static	96 hours	No data	6.7 mg/L (n.c.)	No data	related to Form-aldehyde; no GLP	FA Doc. III A 7.4.1.1/02_ HCHO Klimisch 2
Non guideline study	*Ictalurus melas* (black bullhead)	mortality	static / flow-through	96 hours	No data	25 - 69 mg/L (n.c.)	No data	related to Form-aldehyde; test with 9 fish species; no GLP	FA Doc. III A 7.4.1.1/03_ HCHO Klimisch 2
German UBA test directive	*Danio rerio* (zebra fish)	mortality	static	96 hours	30 mg/L (n.c.)	41 mg/L (n.c.)	50 mg/L (n.c.)	related to Form-aldehyde; no GLP	FA Doc. III A 7.4.1.1/04 Klimisch 4
Non guideline study	*Morone saxatilis* (striped bass)	mortality	static	96 hours	No data	1.8 mg/L 5.0 mg/L **5.7 mg/L** 4.0 mg/L (n.c.)	No data	related to Form-aldehyde; 4 tests performed under different salinity conditions; no GLP	**FA Doc. III-A 7.4.1.1/05**_ HCHO **Klimisch 2**
Comparable to OECD 203	*Pimephales promelas*	mortality	flow-through	96 hours	No data	24.1 mg/L (measured)	No data	related to Form-aldehyde; no GLP	**FA Doc. III A 7.4.1.1/06**_ HCHO **Klimisch 1**

Long-term toxicity to fish

Chronic toxicity of Formaldehyde toward fish *(Danio rerio)* was investigated in two studies, which were both considered as additional information. In the study comparable to OECD Guideline 212 the lowest EC_{50} was obtained with 6.9 mg/L, but test duration (6 days) was shorter than recommended by the guideline. In the second study a NOEC was reported with 48 mg/L.

Short-term toxicity to aquatic invertebrates (cf. **Formaldehyde Doc. III-A 7.4.1.2/03_HCHO**)

Acute toxicity towards invertebrates was tested with the cladocerans *Daphnia magna* and *Daphnia pulex*. Further studies using a number of invertebrate species from a wide array of taxa are reported. The lowest reliable 48h-EC_{50} for invertebrates is **5.8 mg/L** (*D. pulex*).

Table 5.3.2.1-2 Acute toxicity of Formaldehyde to invertebrates (ref. to Formaldehyde Core Dossier Table 4-8)

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			design	duration	EC_0	EC_{50}	EC_{100}		
DIN 38412 Teil11	*Daphnia magna*	immobility	static	24 hours	11.6 mg/L (n.c.)	14.7 mg/L (n.c.)	18.6 mg/L (n.c.)	related to Form-aldehyde	FA Doc. III A 7.4.1.2/01_ HCHO
OECD 202	*Daphnia magna*	immobility	static	48 hours	No data	29 mg/L (n.c.)	No data	related to Form-aldehyde	FA Doc. III A 7.4.1.2/02_ HCHO
OECD 202	*Daphnia pulex*	immobility	static	48 hours	1.9 mg/L (n.c	**5.8 mg/L (n.c.)**	16.8 mg/L (n.c.)	related to Form-aldehyde	**FA Doc. III A 7.4.1.2/03_ HCHO**

Long-term toxicity to aquatic invertebrates

One chronic toxicity study according to OECD guideline 211 with *Daphnia magna* is available for Formaldehyde. In this study a **21 days NOEC of 1.04 mg/L**, based on the age of the first reproduction was found. This study is considered as key study.

Short- and long-term toxicity to algae (cf. **Formaledhyde Doc. III-A 7.4.1.3**)

Algal toxicity was found to be in the same order of magnitude with a **mean E_rC_{50} (72h) of 5.7 mg/L**.

There are no chronic data available.

Table 5.3.2.1-3 Growth inhibition of Formaldehyde on algae (ref. to Formaldehyde Core Dossier Table 4-9)

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			design	duration	NOE_rC	E_bC_{50} [1]	E_rC_{50} [2]		
OECD 201 1st test	*Desmodesmus subspicatus*	cell multiplication inhibition	static static	72 hours	No data	3.48 mg/L (n.c.)	**4.89 mg/L (n.c.)**	related to Form-aldehyde	**FA Doc. III A 7.4.1.3_HCHO**
OECD 201 2nd test					No data	4.4 mg/L (n.c.)	**6.61 mg/L (n.c.)**	related to Form-aldehyde	**FA Doc. III A 7.4.1.3_HCHO**
					Mean		**5.7 mg/L**		

[1] calculated from the area under the growth curve; [2] calculated from growth rate

Conclusion:

Acute aquatic studies demonstrate that Formaldehyde exhibits acute toxicity against fish, invertebrates and algae.

5.3.2.2 Aquatic toxicity - Morpholine

For Morpholine only data from literature are available. To establish the aquatic toxicity of Morpholine, a data search was conducted in a review (WHO, 1996) and a database (HSDB, 2007), both peer reviewed. In Mor Doc. III-A 7.4 Additional Information, an overview is presented on the relevant test results of this data search. Additionally for some literature data single Doc. III-As were submitted. The Klimisch scores are those given for the single studies, for which generally very poor data are presented. However having in mind that all presented data are peer reviewed and applying a weight of evidence approach including all data presented in the two sources reliable results were identified.

Short-term toxicity to fish **(**cf. Mor Doc. III-A 7.4.1.1/02)

The acute toxicity of selected amines, among them Morpholine, were tested on aquatic organisms (cf. Mor Doc. III-A 7.4.1.1/02). Results in hard water (320 mg $CaCO_3$/L) show that the LC_{50} is higher than the LC_{50} in soft water (20 mg $CaCO_3$/L). Results of the soft water were not statistically treated due to the wide pH range of the amines dissolved in the unbuffered solution. Results are therefore treated purely indicative. Due to the limited information given, data can be used only supportively. In a second study (cf. Mor Doc. III-A 7.4.1.1/01) the acute toxicity of Morpholine was determined for Hawaiian marine fishes (*Gambusia affinis*, *Chelon engeli*). At 100 mg Morpholine/L all fishes (*Chelon engeli*) were alive, whereas at 320 mg Morpholine/L all organisms died. Due to the steep dose-response curve no LC_{50} value could be calculated. Instead the 96h median tolerance limit of Morpholine was indicted, which is in the range of 100-180 ppm Morpholine/L. At 180 ppm 60% mortality was observed.

All presented studies show deficiencies and no single key study could be identified. However, the results from the single studies confirm each other in that Morpholine is only toxic to fish in concentrations >100 mg/L. Therefore it was concluded in a weight of evidence approach that the results of all studies together are reliable. The LC_{50} of 180 mg/L from Mor Doc. III-A 7.4.1.1/02 was quoted the most reliable value.

Table 5.3.2.2-1 Acute toxicity of Morpholine to fish

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**	**Remarks**	**Reference/ Reliability**
			design	**duration**	**LC_{50}**		
APHA (1965)	*Chelon engeli*	Mortality	static	96 h	TLm* 100-180 ppm	nominal conc., marine	Doc. III-A 7.4.1.1/01_morpholine, Klimisch 3
IRSA (1973)	*Oncorhynchus mykiss*	Mortality	static	96 h	Hard water: 380 mg/L (95% c.I. 375 – 460)	measured conc., freshwater	Doc III-A 7.4.1.1/02_morpholine, Klimisch 3
IRSA (1973)	*Oncorhynchus mykiss*	Mortality	static	96 h	Soft water: 180 mg/L	measured conc., freshwater	Doc III-A 7.4.1.1/02_morpholine, Klimisch 3

* It was not possible to calculate a LC_{50} value, instead an estimate of this value was used, the 96 h median tolerance limit (TLm), at 180 ppm 60% mortality was observed.

Long-term toxicity to fish

Chronic studies on fish are not available.

Short-term toxicity to aquatic invertebrates

Three literature citations were submitted on the acute toxicity to *Daphnia magna*, with 24h EC_{50} values of 100 mg/L (cf. Doc. III-A 7.4.1.2_morpholine, Doc. IV-A 7.4.1.2), 101 mg/L (cited in Doc. III-A 7.4_morpholine Effects on aquatic organisms - Additional information, Doc. IV-A 7.1.3, WHO 1996) and 119 mg/L (cited in Doc. III-A 7.4_morpholine Effects on aquatic organisms - Additional information, Doc. IV-A7.4.1.2). Results are summarized in Table 5.3.2.2-2. There are no data available for an exposure period of 48h.

Again all presented studies show deficiencies, therefore no single key study could be identified. However, the results of the single studies confirm each other in that the EC_{50} values for Morpholine are all in the range of 100 – 119 mg/L. In the fish study (Doc. III-A 7.4.1.1/02_morpholine, study A 7.4.1.1/02) the test

substance was shown to be stable. Therefore it was concluded as a weight of evidence that the results of all studies together are reliable.

Table 5.3.2.2-2 Acute toxicity of Morpholine to invertebrates

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**			**Remarks**	**Reference/ Reliability**
			design	**duration**	**EC_0**	**EC_{50}**	**EC_{100}**		
AFNOR, Norme Expérimentale N.F.T 90-301 (1974);	*Daphnia magna*	Mobility	static; 18 – 22°C; pH 7.9 ± 0.3	24 h	-	119 mg/L c.I. (112 – 117 mg/L)	-	Measured conc. freshwater	Cited in Doc. III-A 7.4_morpholine, Doc. IV-A 7.4.1.2 Klimisch 3
	Daphnia magna	Mobility	Static; 20°C,pH 8± 0.2	24 h	68 mg/L	101 mg/L c.I. (83 – 122 mg/L)	260 mg/L		Cited in Doc. III-A 7.4 _morpholine, Doc. IV A 7.1.3 WHO, 1996 Klimisch 3
Non-Guideline study	*Daphnia magna*	Mobility	static	24 h	16 mg/L (n.c.)	100 mg/L (n.c.)	500 mg/L (n.c.)[1]	nominal concentr.	Doc. III-A 7.4.1.2_morpholine, Doc IV-A 7.4.1.2 Klimisch 3

Long-term toxicity to aquatic invertebrates

Chronic studies on daphnids are not available.

Short- and long-term toxicity to algae (cf. Doc. III-A 7.4.1.3_morpholine)

The results presented in Doc. III-A 7.4.1.3_morpholine, Doc. IV-A 7.4.1.1/02 show a NOE_rC of 10 mg/L and a 96h E_rC_{50} of 28 mg/L. This study by Calamari et al (1980)[9] used GC FID as analytical method. The culture medium was according to DIN 38412 L33 and ISO 8692 with a $NaHCO_3$ concentration in the test solution of 300 mg/L. Algal growth was evaluated by measuring in vivo the fluorimetric units at 48, 72, 96 hours. Data were extrapolated from the eye fitted empirical curve on log-probability paper with percentages of growth inhibition and the logarithm of concentrations. Data presented in the publication always refer to nominal concentration if the difference between measured and nominal no more than 10%. Results of the controls were 10^6 cells/mL (mean of six replicates). Although the study is performed according to national guideline, only few data are presented in the publication, so that the validity criteria cannot fully be verified.

In another paper (cited in Doc. III-A 7.4_morpholine Effects on aquatic organisms - Additional information, Doc. IV-A7.1.3, WHO 1996) the effects of varying growth medium composition on the toxicity of Morpholine to three different algae species were studied. Due to the limited information given, data can only be used supportively. The toxic effects, which depend on the species and the test mediums used are summarized in Table 5.3.2.2-3.

All presented studies show deficiencies and no single key study could be identified. The E_rC_{50} of 28 mg/L and the NOE_rC of 10 mg/L (cf. Doc. III-A 7.4.1.3_morpholine, Doc. IV-A 7.4.1.1/02), are the lowest values

[9] Calamari D, Da Gasso R, Galassi S, Provini A, Vighi M (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere 9, 753-762

based on growth rate. In the fish study (Doc III-A 7.4.1.1/02_morpholine, study A 7.4.1.1/02) the test substance was shown to be stable. The NOE_rC value is within the lowest NOEC values of four different species, although effect values based on growth rate normally show less toxicity than effect values based on biomass. Therefore the results based on growth rate were quoted reliable.

The **NOE_rC value of 10 mg/L** was quoted to be reliable seen the results of all available data in a weight of evidence approach.

Table 5.3.2.2-3 Growth Inhibition of Morpholine on algae

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**			**Remarks**	**Reference/ Reliability**
			design	**duration**	**NOE_rC**	**E_bC_{50}[2]**	**E_rC_{50}[3]**		
	Chlorella vulgaris	Growth rate	22°C	24-144 h	80 mg/L	-	-	OECD medium; N:P ratio 14:1	Cited in Doc. III A 7.4_morpholine, Doc. IV A 7.1.3 WHO, 1996 Klimisch 3
	Selenastrum subspicatus (current name: *Desmodesmus subspicatus)*	Biomass	22°C	24-120 h	5[4] mg/L	-	-	OECD medium; N:P ratio 14:1	Cited in Doc. III A 7.4_morpholine,, Doc. IV A 7.1.3 WHO, 1996 Klimisch 3
	Selenastrum capricornutum (current name: *Pseudokirch-neriella subcapitata)*	Biomass	22°C	24-120 h	50[4] mg/L	-		OECD medium; N:P ratio 14:1	Cited in Doc. III A 7.4_morpholine,, Klimisch 3
EPA (1971)	*Selenastrum capricornutum*	Growth rate	static	96 h	10[1] mg/L (n.c.)	No data	28[1] mg/L (n.c.)[3]	nominal concentr.	Doc. III-A 7.4.1.3_morpholine,, Doc. IV-A 7.4.1.1/02 Klimisch 3

[1]equivalent to GC-FID measured concentration ± 10%, [2] calculated from the area under the growth curve; [3] calculated from growth rate, [4] E_bC_0

5.3.2.3 Summary and Conclusion:

Several studies on the acute toxicity of Morpholine to fish were conducted. In a non-exhaustive search test results on 8 different freshwater and marine species with effect values between 100 mg/L (96h LC_{50}, *Chelon engeli*) and >1000 mg/L (96h LC_{50}, *Danio rerio*) were found (cf. Doc. III-A 7.4_morpholine Additional information). The lowest effect value expected to be reliable was obtained in a study with the marine fish *Chelon engeli* as test organism (cf. Doc. III-A 7.4.1.1/01_morpholine). In this study a 96h TLm of 100 - 180 mg/L was determined. Results of analytical measurements during the test on *Oncorhynchus mykiss* revealed that the Morpholine concentrations deviated by less than 10% of the nominal values (cf. Doc. III-A 7.4.1.1/02_morpholine). Chronic studies on fish were not located. There were three tests conducted on the acute toxicity to *Daphnia magna*, with 24 h-EC_{50} values between 100 and 119 mg/L (cf. Doc. III-A 7.4_morpholine Additional information, Doc. IV-A 7.4.1.2 and Doc IV-A 7.1.3 WHO, 1996 and Doc. III-A 7.4.1.2_morpholine, Doc IV-A 7.4.1.2). Chronic studies on daphnids were not located. The most sensitive

trophic level tested is algae. $NOE_{b+r}C$ or EC_{b+r0} values between 4.1 and 100 mg/L were obtained in tests with 4 different species (cf. Doc. III-A 7.4_morpholine Additional information). A growth inhibition test with *Pseudokirchneriella subcapitata* (former *Selenastrum capricornutum)* according to EPA (1971) guideline resulted in a 96 h-NOE_rC of 10 mg/L (cf. Doc. III-A 7.4.1.3_morpholine, Doc. IV-A 7.4.1.1/02). Other available tests are considered to be less relevant because of the very long test periods (up to 192h) or because biomass production was determined as only endpoint.

5.3.3 Aquatic toxicity – Comparison of MBM with products of hydrolysis

In Tables 5.3.3-1 and -2 acute and chronic comparable effect data for N,N'-Methylenebismorpholine and its hydrolysis products are presented. Results with the same test organisms and the lowest reliable effect value are listed for Formaldehyde and Morpholine, if available, in order to allow a comparison between the toxicity of both compounds.

Table 5.3.3-1 Comparison of acute aquatic toxicity data

Endpoint		**Hydrolysis products of N,N'-Methylenebis-morpholine**	**Formaldehyde**	**Morpholine**
Acute	Fish	**96h-LC_{50} >100 mg/L (based on confirmed nominal conc. of Morpholine) ≅ 107 mg/L MBM (calc.) ≅ 18 mg/L Formaldehyde (calc.) (*Oncorhynchus mykiss*)**	**96h-LC_{50} = 5.7 mg/L** (*Morone saxatilis*) (based on nominal conc. of Formaldehyde)	Hard freshwater: 96h-LC_{50} = 380 mg/L (*Oncorhynchus mykiss*) Soft freshwater: 96h-LC_{50} = 180 mg/L (*Oncorhynchus mykiss*) Marine water: 96h-TLm* = 100-180 ppm (*Chelon engeli*)
	Invertebrates	24h-EC_{50} = 71 mg/L (based on confirmed nominal conc. of Morpholine) ≅ 76 mg MBM (calc.) ≅ 12.6 mg Formaldehyde (calc.) (*Daphnia magna*) **48h-EC_{50} = 24 mg/L (based on confirmed nominal conc. of Morpholine) ≅ 26 mg/L MBM (calc.) ≅ 4.3 mg/L Formaldehyde (calc.) (*Daphnia magna*)**	24h-EC_{50} = 14.7 mg/L (Daphnia magna) [No 24h-EC_{50} for Daphnia pulex] 48h-EC_{50} = 29 mg/L (Daphnia magna) **48h-EC_{50} = 5.8 mg/L (Daphnia pulex)**	24h-EC_{50} = 100-119 mg/L (*Daphnia magna*) in 3 different studies No data available for 48h exposure.
	Algae	72h-E_bC_{50} = 4.4 mg/L **96h-E_rC_{50} = 9.5 mg/L (based on confirmed nominal conc. of Morpholine) ≅ 10 mg/L MBM (calc.) ≅ 1.6 mg/L Formaldehyde (calc.) (*Pseudokirchneriella subcapitata*)**	**72h-E_rC_{50} = 4.89 mg/L 72h-E_rC_{50} = 6.61 mg/L Mean value: 5.7 mg/L (*Desmodesmus subspicatus*)**	96h-E_rC_{50} = 28 mg/L (*Pseudokirchneriella subcapitata*, former *Selenastrum capricornutum*)

* It was not possible to calculate a LC_{50} value, instead an estimate of this value was used, the median tolerance limit (TLm). Calc., calculated. The active substance can be characterised by the releasable total formaldehyde content which is typically 16.7% (see **Doc. III-A 4.1/02**, Study IV-A 4.1/02).

Table 5.3.3-2 Comparison of chronic aquatic toxicity data

Endpoint		**Hydrolysis products of N,N'-Methylenebis-morpholine**	**Formaldehyde**	**Morpholine**
Chronic	Fish	Not available	6d-LC_{50} = 6.9 mg/L (*Danio rerio, sac-fry stages*) 28d-NOEC = 48 mg/L *(Oryzias latipes)*	Not available
	Invertebrates	**21d-NOEC = 5 mg/l (based on confirmed nominal concentration of MBM) ≅ 4.7 mg Morpholine ≅ 0.8 mg Formaldehyde (*Daphnia magna*)**	**21d-NOEC = 1.04 mg/l *(Daphnia magna)***	Not available
	Algae	**72h-NOErC = 2.0 mg/L (based on nominal confirmed conc. of Morpholine) ≅ 2.1 mg MBM ≅ 0.36 mg Formaldehyde (*Pseudokirchneriella subcapitata*)**	Not available	96h-NOE_rC = 10 mg/L (*Pseudokirchneriella subcapitata*, former *Selenastrum capricornutum*)

In the media of aquatic toxicity tests, N,N'-methylenebismorpholine is expected to be hydrolysed completely to an aldehyde (Formaldehyde) and Morpholine. Therefore, the observed toxicity should be caused by the hydrolysis products.

The overview reveals that Formaldehyde has a higher acute toxicity than Morpholine (fish, invertebrates), the values for the parent compound N,N'-methylenebismorpholine are between those of the hydrolysis products. Hydrolysis of 1 g N,N'-methylenebismorpholine leads to the formation of 0.16 g Formaldehyde and 0.94 g Morpholine. Comparison with the effect data in the table indicates that the toxicity of N,N'-methylenebismorpholine is primarily determined by its Formaldehyde content. Only limited comparison between the chronic toxicity of the parent compound and the metabolites is possible since relevant data are missing. The few available chronic data indicate the same situation as for the acute toxicity studies, with increasing toxicity from Morpholine to MBM and Formaldehyde.

5.3.4 Other aquatic organisms (including sediment)

5.3.4.1 Inhibition of microbial activity (aquatic) – MBM (cf. MBM Doc. III-A 7.4.1.4)

The toxicity of the test material OS 157340 (=MBM, purity 98% w/w) towards bacteria was tested according to OECD Guideline 209 (cf. MBM Doc. III-A 7.4.1.4) by determining the inhibition of respiration in sludge samples from a biological treatment plant receiving predominantly domestic sewage. During the test N,N'-methylenebismorpholine hydrolysed, therefore the effect data are not attributable to MBM, but rather to the hydrolysis products Formaldehyde and Morpholine. In the tests, the 3h EC_{50} was established at a concentration of 340 mg MBM/L (calculated ≅317.6 mg Morpholine/L and calculated ≅12.6 mg Formaldehyde/L). The NOEC was determined to be 32 mg MBM/L (calculated ≅29.9 mg Morpholine/L and calculated ≅5.3 mg Formaldehyde/L) based on the nominal concentration of the test material. Formaldehyde and Morpholine were not determined analytically.

Table 5.3.4.1-1 Inhibition of microbial activity (aquatic) by the hydrolysis products of N,N'-methylenebismorpholine

Guideline /Test method	Species / Inoculum	Endpoint / Type of test	Exposure		Results			Remarks	Reference/ Reliability
			design	duration	NOEC	EC_{50}	EC_{80}		
OECD 209	Activated sludge, municipal	Inhibition of respiration	static	3 h	**32 mg/L**	340 mg/L	n.d.	nominal conc., GLP	**MBM Doc. III-A-7.4.1.4,** Klimisch 1

5.3.4.2 Inhibition of microbial activity (aquatic) – Products of hydrolysis Formaldehyde (cf. Formaldehyde Core Dossier)

The acute toxicity of Formaldehyde towards bacteria was investigated in two studies (cf. **Formaldehyde Core Dossier Table 4-10)**. The test according to OECD Guideline 209, determining the inhibition of respiration in a sewage sludge sample, resulted in an **EC_{50} of 20.4 mg/L.**

Table 5.3.4.2-1 Formaldehyde: Inhibition of aquatic microbial activity **(**Formaldehyde Core Dossier Table 4-10)

Guideline / Test method	Species / Inoculum	Endpoint / Type of test	Exposure		Results			Remarks	Reference
			design	duration	EC_{10}	EC_{50}	EC_{90}		
OECD 209	activated sludge	respiration inhibition	Static	3 h	No data	**20.4 mg/L (n.c.)**	No data	related to form-aldehyde	**FA Doc. III-A7.4.1.4/01**
Offhaus K (1973) Münch Beitr Abwasser-, Fisch-, Flussbiol 24, 169-196	mixed bacterial culture obtained from settled municipal wastewater	respiration inhibition associated with peptone degradation	Static 20±1°C pH 8.4	120 h	14.7 mg/L (n.c.)	34.1 mg/L (n.c.)	78.9 mg/L (n.c.)	related to form-aldehyde	FA Doc. III-A7.4.1.4/02

Morpholine

The effect of Morpholine to the respiration of activated sludge was tested according to OECD guideline 209 (cf. Doc. III-A 7.4.1.4_morpholine). Morpholine was applied in only one concentration (1000 mg/L). This test concentration resulted in 15% inhibition of respiration and in 10-20% inhibition of dehydrogenase activity. No dose-response curve including an EC_{50} or NOEC value could be derived. No blank controls were conducted. The documentation of the materials and methods section of the study is rather limited. Therefore the study as such is rated as Klimisch category 3.

In addition to the guideline study, growth inhibition on 4 strains of *Pseudomonas* and toxicity threshold on *Pseudomonas putida* and on *Microcystis aeruginosa* were studied (cf. Doc. III-A 7.4_morpholine – Additional Information) in 3 tests, which resulted in effect values of 8700 mg/L (NOE_rC), 310 mg/L (16h TT) and 1.7 mg/L (192h TT). However, these tests were conducted according to methods which are nowadays considered to be not valid.

Additional tests on 3 different protozoan species resulted in effect values of 12 mg/L (72h TT), 18 mg/L (48h TT) and 815 mg/L (20h TT).

Having in mind all results with 4 different strains of *Pseudomonas* and with *Microcystis aeruginosa* and the very long test durations, which lead to the low threshold values, the results of the study performed according to OECD guideline 209 can be quoted reliable in a weight of evidence approach.

5.3.4.3 Inhibition of microbial activity (aquatic) – Comparison of MBM with products of hydrolysis

Table 5.3.4.3-1 Comparison of data for inhibition of microbial activity (aquatic)

N,N'-Methylenebismorpholine	**Formaldehyde**	**Morpholine**
3 h-EC_{50} = 340 mg/L 3 h-NOEC = 32 mg/L (Respiration inhibition of sludge)	3 h-EC_{50} = 20.4 mg/L (Respiration inhibition of sludge)	30 min-EC_{15} = 1000 mg/L (Respiration inhibition of sludge)

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

Aquatic Acute 1:

All available acute L(E)C_{50} values for MBM, Formaldehyde and Morpholine for all three trophic levels are >1 mg/L. The lowest L(E)C_{50} values available are the E_rC_{50} (algae) and LC_{50} (fish) for Formaldehyde with 5.7 mg/L. Therefore no classification with Aquatic Acute 1 is necessary.

➔ **No classification**

Studies used:

MBM:

- MBM Doc. III-A 7.4.1.1: Lubrizol Corporation (2001), OECD 203, OS 157340: Acute Toxicity to Rainbow Trout (*Oncorhynchus Mykiss)* -> **(96h) LC_{50} (fish, calculated, based on measured concentrations of Morpholine) =107 mg/L**
- MBM Doc. III-A 7.4.1.2: Lubrizol Corporation (2001), OECD 202, OS 157340: Acute Toxicity to *Daphnia Magna* -> **(48h) EC_{50} (crustacean, calculated, based on measured concentrations of Morpholine) =26 mg/L**
- MBM Doc. III-A 7.4.1.3: Lubrizol Corporation (2001), OECD 201, OS 157340: Algal Inhibition Test -> **(96h) E_rC_{50} (algae, calculated, based on measured concentrations of Morpholine) =10 mg/L**

Formaldehyde: (for details see Formaldehyde Core Dossier)

- FA Doc. III-A 7.4.1.1/05_HCHO **-> (96h) LC_{50} (fish) =5.7 mg/L**
- FA Doc. III-A 7.4.1.2/03_HCHO -> **(48h) EC_{50} (crustacean) = 5.8 mg/L**
- FA Doc. III-A 7.4.1.3_HCHO -> **(72h) E_rC_{50} (algae) = 5.7 mg/L (mean)**

Morpholine:

- Doc. III-A 7.4.1.1/01_morpholine: McCain J,C. & Peck J.M. Jr. (1976), APHA The toxicity of selected chemicals used in power generating stations to Hawaiian fishes NOAA. Washington, DC, US Department of Commerce, National Technical Information Service, 23 pp (NTIS No PB262437) -> **(96h) TLm (fish) =100-180 ppm**
- Doc. III-A7.4.1.1/02_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M. (1980), IRSA Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 -> **(96h) LC_{50} (fish, hard water) =380 mg/L, (fish, soft water) =180 mg/L**
- Doc. III-A7.4.1.2_morpholine: Bringmann G., Kuehn R., (1977) Befunde der Schadwirkung wasser-gefährdender Stoffe gegen *Daphnia magna*. Z Wasser Abwasser-Forsch 10, Nr. 5/77, 161-166 -> **24h EC_{50} (crustacean) =119 mg/L**
- Doc. III-A7.4.1.2_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M., (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 -> (**24h) EC_{50} (crustacean) =100 mg/L**

- Doc. III-A7.4.1.3_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M., (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 **-> (96h) E_rC_{50} (algae) =28 mg/L**

Aquatic Chronic Categories:

For MBM 2 long-term NOECs are available for crustacean and algae, which are both >1 mg/L. For fish an acute LC_{50} >100 mg/L is available and MBM is readily biodegradable; additionally a calculated log K_{ow} of -1.53 and a calculated BCF_{fish} of 1.41 L/kg are available. On the basis of these data no classification for any of the chronic categories is needed for MBM.

There is only one reliable chronic NOEC value available (>1 mg/L) for Formaldehyde from crustacean. For fish and algae EC_{50} values >1 mg/L are available, which in combination with ready biodegradability, a measured log K_{ow} of 0.35 and a calculated BCF_{fish} of 0.396 L/kg doesn't lead to any classification.

Morpholine shows a chronic NOEC for algae of >1 mg/L. For fish and crustacean there are acute $L(E)C_{50}$ ≥100 mg/L available. In addition Morpholine is readily biodegradable; it has a measured log K_{ow} =-0.86 and a measured BCF <2.8 L/kg. Again these data don't lead to any classification for Morpholine.

Therefore no classification for hazards to the aquatic environment is proposed for MBM, since neither the available data on MBM itself, nor the data on its hydrolysis products fulfill the criteria.

Aquatic Chronic 1, 2, 3 and 4:

➔ **No classification**

Studies used:

MBM:

- MBM Doc. III-A 7.1.1.2.1**:** Lubrizol Corporation (2001), OECD 301 B OS 157340: Assessment of ready biodegradability; CO_2 Evolution Test -> **93% degradation in 28 days**
- MBM Doc. III-A 3: Partition coefficient of MBM, (Estimation with EPI Suite) -> **log K_{ow} =-1.53**
- Calculation according to TGD on Risk Assessment (EC 2003, part II, chapter 3, p. 125) -> **BCF_{fish} (calculated for a log Kow of 1) =1.41 L/kg**
- MBM Doc. III-A 7.4.1.1: Lubrizol Corporation (2001), OECD 203, OS 157340: Acute Toxicity to Rainbow Trout (*Oncorhynchus Mykiss)* -> **(96h) LC_{50} (fish, calculated, based on measured concentrations of Morpholine) =107 mg/L**
- MBM Doc. III-A 7.4.3.4: Lubrizol Corporation (2007), Study on the Chronic Toxicity towards Daphnia of „ST-1" according OECD-Guideline No. 211 *(Daphnia magna* Reproduction Test) **-> (21 days) NOEC (crustacean, calculated, based on measured concentrations of Formaldehyde) =4.7 mg/L**
- MBM Doc. III-A 7.4.1.3: Lubrizol Corporation (2001), OECD 201, OS 157340: Algal Inhibition Test -> **(72 h) NOE_rC (algae, calculated, based on measured concentrations of Morpholine) =2.1 mg/L**

Formaldehyde:

- FA Doc. III-A 7.1.1.2/04_HCHO**:** OECD 301 A -> **readily biodegradable**

- FA Doc. III-A 3_HCHO: Hansch et al. (1995), Sangaster (1989), in accordance with 92/69/EEC A.9, Shake-Flask Method, Partition coefficient of Formaldehyde -> measured **log K_{ow} =0.35**
- Calculation according to TGD on Risk Assessment -> **$BCF_{fish.\ calculated}$ =0.396**
- FA Doc. III-A 7.4.1.1/05_HCHO **-> (96h) LC_{50} (fish) =5.7 mg/L**
- FA Doc. III-A 7.4.1.2/03_HCHO -> **(21 days) NOEC (crustacean) = 1.04 mg/L**
- FA Doc. III-A 7.4.1.3_HCHO -> **(72h) E_rC_{50} (algae) = 5.7 mg/L (mean)**

Morpholine:

- Doc. III-A 7.1.1.2.1/01_morpholine**:** Strotmann U.J., Weberruß U., & Bias W.R. (1993), OECD 301E Degradation of morpholine in several biodegradation tests and in wastewater treatment plants. Chemosphere, Vol.26 (9) 1729-1742 -> **90% degradation in 28 days**
- Doc. III-A 3_morpholine: Hansch et al. (1995) Partition coefficient of Morpholine -> **measured log K_{ow} =-0.86**
- Doc. III-A 7.4.2_morpholine -> HSDB, (2007), Morpholine– Environmental Fate & Exposure Data, Phys-Chem Data from Hazardous Substances Data Bank, state June 15, 2007, last revision date 24.06.2005 7p -> **experimental BCF_{fish} <2.8 L/kg**
- Doc. III-A 7.4.1.1/01_morpholine: McCain J,C. & Peck J.M. Jr. (1976), APHA The toxicity of selected chemicals used in power generating stations to Hawaiian fishes NOAA. Washington, DC, US Department of Commerce, National Technical Information Service, 23 pp (NTIS No PB262437) -> **(96h) TLm (fish) =100-180 ppm**
- Doc. III-A7.4.1.1/02_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M. (1980), IRSA Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 -> **(96h) LC_{50} (fish, hard water) =380 mg/L, (fish, soft water) =180 mg/L**
- Doc. III-A7.4.1.2_morpholine: Bringmann G., Kuehn R., (1977) Befunde der Schadwirkung wassergefährdender Stoffe gegen *Daphnia magna*. Z Wasser Abwasser-Forsch 10, Nr. 5/77, 161-166 -> **(24h) EC_{50} (crustacean) =119 mg/L**
- Doc. III-A7.4.1.2_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M., (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol 9, 753-762 -> **(24h) EC_{50} (crustacean) =100 mg/L**
- Doc. III-A7.4.1.3_morpholine: Calamari D., Da Gasso R., Galassi S., Provini A., Vighi M., (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 -> **(96h) NOE_rC (algae) =10 mg/L**

Hazards to the ozone layer:

On the basis of low vapour pressure, low Henrys Law constants and rapid degradation through reaction with hydroxyl radicals for MBM as well as for its hydrolysis products there are no indications for danger to the ozone layer.

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

No classification for hazards to the aquatic environment and to the ozone layer is proposed for MBM, since neither the available data on MBM itself, nor the data on its hydrolysis products fulfill the criteria.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

4-(morpholin-4-ylmethyl)morpholine (MBM) hydrolyses in water to formaldehyde and morpholine. It is assumed that the toxicity of MBM is related to the formaldehyde release.

Degradation

The DS proposed to consider MBM as rapidly degradable. The basis for this proposal was that the OECD 301B test results show that MBM and its hydrolysis products are readily biodegradable (93% degradation based on CO_2 evolution within 28 days).

Aquatic Bioaccumulation

The DS proposed that MBM does not meet the CLP criteria for bioaccumulation. The basis for this proposal was an estimated log $P_{ow} \leq 0.3$ and no significant bioaccumulation potential is expected for MBM.

Acute Toxicity

The DS proposed to not classify MBM as acutely hazardous to the aquatic environment. The basis for this proposal was that the acute toxicity of N,N'-methylenebismorpholine to aquatic organisms was tested in several studies covering all three trophic levels (fish, daphnia, and algae). The test solutions were prepared some hours before the test organisms were introduced, ensuring complete hydrolysis of the test substance OS 157340 (=MBM, purity 98% w/w). In all acute tests the hydrolysis product morpholine was monitored, in contrast to formaldehyde, which was not analysed. The test organisms were therefore exclusively exposed to the hydrolysis products, instead of the parent substance. Monitoring revealed that the test substance (measured as morpholine) was stable over the test period. The nominally confirmed concentrations based on morpholine were used to derive the effect values for the test substance.

All available acute $L(E)C_{50}$ values for MBM for all three trophic levels were >1 mg/L. The lowest $L(E)C_{50}$ values available was for algae; a 96h-ErC_{50} = 9.5 mg/L (based on nominal conc. of morpholine ≈ 10 mg/L MBM (calc.) ≈ 1.6 mg/L formaldehyde) (calculated)

Chronic Toxicity

The DS proposed to not classify MBM as chronically hazardous to the aquatic environment. The basis for this proposal was that long-term NOECs were available for crustaceans and algae, which were both >1 mg/L. For algae a 72h-NOE_rC of 2.0 mg/L (based on a nominal concentration of morpholine ≈ 2.1 mg MBM ≈ 0.36 mg formaldehyde) was derived. A study on chronic fish toxicity with N,N'-methylenebismorpholine as the test substance is not available.

Comments received during public consultation

One MSCA CA commented on the ENV part of the classification dossier and requested further evaluation of an algae study on formaldehyde, which is one hydrolysis degradation product of MBM. The algae study is only available as a literature publication without any raw data or concentration-response curves. Only the 72h-E_rC_{50} of 5.7 mg/L was published. Consequently, the literature publication does not allow the derivation of a NOE_rC, nor an E_rC_{10} or an E_rC_{20} and no assessment against the chronic classification criteria for formaldehyde.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to assess MBM as being rapidly degradable.

Aquatic Bioaccumulation

RAC agrees with the DS that MBM does not fulfil the criteria on aquatic bioaccumulation.

Acute Toxicity

RAC agrees with the dossier submitter to not classify MBM as acutely hazardous to the aquatic environment.

Chronic Toxicity

RAC agrees with the dossier submitter to not classify MBM as chronically hazardous to the aquatic enviroment.

6 OTHER INFORMATION

Not available

7 REFERENCES

LIST OF STUDIES FOR THE ACTIVE SUBSTANCE 4-(morpholin-4-ylmethyl)morpholine

Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
A3.1.1	2001	OS 157340: Determination of General Physico-chemical Properties Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No.525/335 GLP, unpublished	Y	Lubrizol
A3.1.3	2007	Determination of the Density of CONTRAM™ ST-1. Lubrizol Industrial Additives, Hamburg July 4, 2007 No GLP, unpublished	Y	Lubrizol
A3.2a	2001	OS 157340: Determination of Vapour Pressure Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No. 525/336 GLP, unpublished	Y	Lubrizol
A3.2b	2005	Estimation of physical chemical properties of N,N-Methylenebismorpholine using EpiSuite 3.12 GLP not applicable, published	Y	Lubrizol
A3.4/01	2007	UV Spectrum of CONTRAM™ ST-1. Lubrizol Metalworking Additives, Spartanburg, SC, USA, July 3, 2007 No GLP, unpublished	Y	Lubrizol
A3.4/02	2007	Determination of the Infrared (IR) Spectrum of CONTRAM™ ST-1. Lubrizol Industrial Additives, Hamburg 17.12.2007 No GLP, unpublished	Y	Lubrizol
A3.4/04	2007	Mass-Spectrum Prof. Dr. Lothar Weber, University of Bielefeld, Department of Chemistry. 09.07.2007 GLP not applicable, unpublished	Y	Lubrizol
A3.4/05		1-H Spektren	Y	Lubrizol
A3.4/06		13-C Spektren	Y	Lubrizol
A3.6b	2007	Determination of the pH-Value of CONTRAM™ ST-1. Lubrizol Industrial Additives, Hamburg July 4, 2007 No GLP, unpublished	Y	Lubrizol
A3.6a	2006	Estimation of the dissociation constants of N,N-Methylolmorpholine by using QSAR ACD/pKa DB, Product Version 10.01, 8.12.2006 GLP not applicable, unpublished	Y	Lubrizol

Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
A3.7a	2006	Determination of the Solubility Range of CONTRAM™ ST-1: N,N'-methylene-bismorpholine (CAS# 5625-90-1) in n-Heptane Using a Turbidimetric Method. Lubrizol Metalworking Additives, January 13, 2006 No GLP, unpublished	Y	Lubrizol
A3.7b	2007	Solubility of CONTRAM™ ST-1, N,N'-methylenebismorpholine (CAS# 5625-90-1) in various organic solvents. Lubrizol Metalworking Additives, June 29, 2007 No GLP, unpublished	Y	Lubrizol
A3.10	2007	Safety-related evaluation of the thermal stability of "CONTRAM(TM) ST-1 BC 6005 / 100500234". Siemens AG, A&D AS SP PPE IPD Prozess-Sicherheit, Rep. No. PS 20070681-3-Kra No GLP, unpublished	Y	Lubrizol
A3.12	2008	Determination of the Flash Point (COC) of Contram™ ST-1. Lubrizol Industrial Additives, Hamburg February 12, 2008 No GLP, unpublished	Y	Lubrizol
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A6.1.2	2001	Statement of non performance of dermal toxicity study in the rat. Safepharm Laboratories Ltd., 03 April 2001	Y	Lubrizol
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A6.6.2	2001	OS157340: Chromosome aberration test in CHL cells in vitro. SafePharm Laboratories Ltd., SPL Project No. 525/309 GLP, unpublished	Y	Lubrizol
A6.6.3	2001	OS157340: L5178 TK+/- mouse lymphoma assay. SafePharm Laboratories Ltd., SPL Project No. 525/310 GLP, unpublished	Y	Lubrizol
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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4-(MORPHOLIN-4-YLMETHYL)MORPHOLINE; [MBM]

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Section No / Reference No	**Year**	**Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published**	**Data Protection Claimed (Yes/No)**	**Owner**
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A7.3.1	2005	EPIWIN 3.12 estimation for N,N-Methylenebismorpholine No GLP, published	Y	Lubrizol
A7.4.1.1	2001	OS 157340: Acute Toxicity to Rainbow Trout (*Oncorhynchus Mykiss* Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No.525/342 GLP, unpublished	Y (Exist./First/)	Lubrizol
A7.4.1.2	2001	OS 157340: Acute Toxicity to *Daphnia Magna* Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No.525/343 GLP, unpublished	Y	Lubrizol
A7.4.1.3	2001	OS 157340: Algal Inhibition Test Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No.525/344 GLP, unpublished	Y	Lubrizol
A7.4.1.4	2001	OS 157340: Assessment of the Inhbitory Effect on the respiratipon of activated Sewage Sludge Safepharm Laboratories Ltd, Derby (United Kingdom), SPL Project No.525/346 GLP, unpublished	Y	Lubrizol
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A6.1.4 Additional information	2000	Morpholin Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Gesundheitsschädliche Arbeitstoffe GLP not applicable, published	No	-
A6.1.4 Additional information	2007	Morpholine European Chemical Bureau, European Chemical Substance Information. http://ecb.jrc.it/esis/ non GLP, published	No	-
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A6.1.4 Additional information	1988	Comparative studies of the sensitization potential of morpholine, 2-mercaptobenzothiazole and 2 of their derivatives in guinea pigs. Contact Dermatitis 19: 11-15 non GLP, published	No	-
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A6.1.5 Additional information	2000	Morpholin Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Gesundheitsschädliche Arbeitstoffe GLP not applicable, published	No	-
A6.1.5 Additional information	1988	Comparative studies of the sensitization potential of morpholine, 2-mercaptobenzo-thiazole and 2 of their derivatives in guinea pigs.	No	-

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A6.1.5 Additional information	1996	Morpholine International Programme on Chemical Safety. Environmental Health Criteria 179, Geneva GLP not applicable, published	No	-
A6.2	1978	Excretion and distribution of morpholine in rats. J Fd Hyg Soc Japan 19: 329-334 non GLP, published	No	-
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A6.3-A.6.5 Additional information	1939	The acute and subacute toxicity of morpholine. J Ind Hyg Toxicol 21: 236-245 non GLP, published	No	-
A6.3-A.6.5 Additional information	1987a	Combined chronic toxicity and carcinogenicity studies of morpholine oleic acid salt in B6C3F1 mice. Fd Chem Toxic 25: 569-574 non GLP, published	No	-
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A6.4.3	1984	Subchronic inhalation toxicity of morpholine in rats. Fundam Appl Toxicol 4: 465-472 non GLP, published	No	-
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A6.6.1-A6.6.6 Additional information	2000	Morpholin Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Gesundheitsschädliche Arbeitstoffe GLP not applicable, published	No	-
A6.6.1-A6.6.6 Additional information	1982	Evaluation of morpholine, piperazine and analogues in the LL5178 mouse lymphoma assay and Balb/3T3 transformation assay. The Society of Toxicology, 13th annual meeting of the environmetal mutagen society, Boston, 22-28 February 1982. New York, John Wiley and sons, pp 1-15 non GLP, published	No	-
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A6.7 Additional information	2000	Morpholin Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, Gesundheitsschädliche Arbeitstoffe GLP not applicable, published	No	-
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A6.7 Additional information	1987a	Combined chronic toxicity and carcinogenicity studies of morpholine oleic acid salt in B6C3F1 mice. Fd Chem Toxic 25: 569-574 non GLP, published	No	-
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A6.8.2 Additional information	1984	Subchronic inhalation toxicity of morpholine in rats. Fundam Appl Toxicol 4: 465-472 non GLP, published	No	-
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A7.1.3	1996	Environmental Health Criteria 179 "Morpholine". World Health Organization, Geneva, 1996 GLP not applicable, published	No	-
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A7.4 Additional Information	1996	Environmental Health Criteria 179 "Morpholine". World Health Organization, Geneva, 1996 GLP not applicable, published	No	-
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A7.4.1.1/02	1980	Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol. 9, 753-762 non GLP, published	No	-
A7.4.1.2	1977	Befunde der Schadwirkung wassergefährdender Stoffe gegen *Daphnia magna*. Z Wasser Abwasser-Forsch 10, Nr. 5/77, 161-166 non GLP, published	No	-
A7.4.1.2	1980	Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere Vol 9, 753-762 non GLP, published	No	-
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A7.4.1.4	1993	Degradation of morpholine in several biodegradation tests and in wastewater treatment plants. Chemosphere, Vol. 26 (9) 1729-1742	No	-
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A7.4.2	1996	Environmental Health Criteria 179 "Morpholine". World Health Organization, Geneva, 1996 GLP not applicable, published	No	-
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8 ANNEXES

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