CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)

EC Number: **not applicable**

CAS Number: **not applicable**

Index Number: **not allocated**

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on behalf of

AT Competent Authority

Federal Ministry of Agriculture, Forestry, Environment and Water Management

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1.1-1: Substance identity

Substance name:	Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)
EC number:	Not applicable
CAS number:	Not applicable
Annex VI Index number:	Not allocated
Degree of purity:	UVCB substance
Impurities:	UVCB substance

The substance “Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)” (short: RP 3:2) is a complex mixture prepared by reaction of paraformaldehyde and 2-hydroxy-propylamine.

As an UVCB substance, the active substance is identified by its source and the manufacturing process (e.g., ratio paraformaldehyde and 2-hydroxypropylamine = 3:2, temperature, etc.). The starting materials are paraformaldehyde and 2-hydroxypropylamine

Purity/impurities, additives

The minimum degree of purity cannot be set for the UVCB substance. The active substance is identified by its source and the manufacturing process.

There are no additives in the substance as manufactured.

Current Annex VI entry: No current Annex VI entry.

1.2 Harmonised classification and labelling proposal

Table 1.2-1: The current Annex VI entry and the proposed harmonised classification of Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)

	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. 1A, H317: May cause an allergic skin reaction, Carc. 1B, H350: May cause cancer by inhalation Muta 2, H341: Suspected of causing genetic defects Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Corr. 1B, H314: Causes severe skin burns and eye damage Skin Sens. 1A, H317: May cause an allergic skin reaction, Carc. 1B, H350: May cause cancer by inhalation Muta 2, H341: Suspected of causing genetic defects Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects

Please find below the harmonized classification of the hydrolysis products formaldehyde (CAS Number: 50-00-0) and 2-hydroxypropylamine (CAS Number: 78-96-6) according to the Committee for Risk Assessment RAC (2012)[1] and the CLP Regulation (EC) No. 1272/2008[2], respectively. Please note that the two substances showed no classification regarding physico-chemical properties and environmental effects.

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 2-hydroxypropylamine (ratio 3:2).

[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 1.2-2: The current Annex VI entry and harmonised classification of Formaldehyde and 2-Hydroxypropylamine

	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 %
2-Hydroxypropylamine	
Current entry in Annex VI, CLP Regulation	Skin Corr. 1B, H314: Causes severe skin burns and eye damage

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

Table 1.2-3: 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	conclusive but not sufficient for classification
2.3.	Flammable aerosols	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
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

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
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	conclusive but not sufficient for classification
3.4.	Skin sensitisation	Skin Sens. 1A, H317: May cause an allergic skin reaction		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	Aquatic Chronic 3 H412: Harmful to aquatic life with long lasting effects.	n.a.	currently not classified	n.a.

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
5.1.	Hazardous to the ozone layer				

[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
H412: Harmful to aquatic life with long lasting effects

Precautionary statements:
P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P273: Avoid release to the environment
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:

Skin Corr. Cat 1, H314: Causes severe skin burns and eye damage
Standard rabbit data are available supporting irreversible damage to skin and eyes.

Skin Sens. Cat 1A, H317: May cause an allergic skin reaction
Standard Guinea Pig Maximization Test data are available supporting skin sensitizing effects with intradermal induction concentrations of ≤1% and challenge response rates of ≥60%.

Carc. Cat 1B, H350: May cause cancer & Muta Cat 2, H341: Suspected of causing genetic defects
No carcinogenicity study is available for the substance, but hydrolyses to formaldehyde by dilution and by reaction with biological media is the mode of biocidal action. Hydrolysis studies indicate a DT50 of < 1 hour. It is proposed to read across the classification of formaldehyde to the formaldehyde-releaser based on consideration of total releasable formaldehyde.

Environment:

Acute aquatic toxicity: $L(E)C_{50}$ values between 1 - 100 mg/L; lowest acute value E_rC_{50} (algae) =1.8 mg/L;

Chronic Aquatic toxicity: lowest NOE_rC value for algae =0.5 mg/L, NOEC daphnia = 1.3 mg/L

Fate & behaviour: rapidly degradable; log K_{ow} <4;

Proposed C&L (according to the data summarised above):

CLP:

- No classification with Aquatic Acute 1, since all available acute toxicity values >1 mg/L.
- Classification with **Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects** on the basis of the available chronic NOE_rC value from algae with 0.5 mg/L in combination with rapidly degradable.

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

No current classification and labelling.

2.4.2 Current self-classification and labelling based on DSD criteria

Classification	By the manufacturer
Class of danger	C Corrosive
R phrases	R21/22 Harmful in contact with skin and if swallowed. R34 Causes burns. R52 Harmful to aquatic organisms
S phrases	S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37/39 Wear suitable protective clothing, gloves and eye/face protection. S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S61 Avoid release to the environment. Refer to special instructions/safety data sheets

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

The substance “Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)” (short: RP 3:2) is a complex mixture prepared by reaction of paraformaldehyde and 2-hydroxy-propylamine.

The substance RP 3:2 is an biocidal active substance which has originally been notified under the name “3,3’-methylene-bis(5-methyloxazolidine)” (shortly named MBO) according to Directive 98/8/EC concerning the placing of biocidal products on the market. The evaluation of the dossier has shown that the active substance is a complex reaction mixture (UVCB Substance) with 3,3’-methylene-bis(5-methyloxazolidine) being only one of the constituents. Therefore in consultation with the Commission and the substance manufacturer it has been decided that the active substance notified as 3,3’-methylene-bis(5-methyloxazolidine) should be renamed to reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio 3:2).

As an UVCB substance, the substance is identified by its source and the manufacturing process (e.g., ratio paraformaldehyde and 2-hydroxypropylamine = 3:2, temperature, etc.). The starting materials are paraformaldehyde and 2-hydroxypropylamine, and the process of manufacture is described below.

Formaldehyde generally reacts with terminal OH-groups. In a first step formaldehyde reacts with the OH-group of 2-hydroxypropylamine under formation of 1-(hydroxymethylamino)propan-2-ol which is in equilibrium with 5-methyl-1,3-oxazolidine. This intermediate reacts in dependant of the molar ratio of the starting materials to MBO or the by-product. At a molar ratio paraformaldehyde / 2-hydroxypropylamine = 3:2 mainly N,N’-methylene-bis(5-methyloxazolidine) is formed with the aid of vacuum and energy, while at a molar ratio of 1:1 α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) is the product. The proposed reaction scheme is presented in Figure 1.1-2 in Doc IIA-Confidential of the draft Competent Authority Report attached to IUCLID section 13.

During production of the active substance, additionally some amounts of α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) are formed. As an excess of formaldehyde is present in the reaction mixture and synthesis is conducted at higher temperature and under reduced pressure, the equilibrium is shifted via the intermediate to N,N’-methylene-bis(5-methyloxazolidine). Nevertheless, HPT is present in the product as by-product.

Because of hydrolysis, chromatographical methods or derivatization are not applicable to determine the content of the single constituents.

NMR spectra can be used as fingerprint (qualitative) in order to identify the main organic components of the reaction product.. The composition and identity of the reaction mixture was studied by ^{1}H and ^{13}C-NMR spectroscopy. Besides the signals from the main ingredient MBO, signals from hydrolysis products (i.a. HPT) were observed.

Supporting data has been obtained from ^{13}C-NMR investigations which were performed to characterise the reaction product in more detail. A semi-quantitative determination by ^{13}C-NMR resulted in relative organic carbon contents of some organic constituents of the reaction product. For details see Doc IIA-Confidential of the draft CAR attached to IUCLID section 13. These values give only a rough estimation about the composition of the reaction mixture and the concentration of the minor constituents.

Remark:

As explained above the biocidal active substance has been originally notified as 3,3'-methylene-bis(5-methyloxazolidine)", shortly named MBO. As a result of the evaluation the substance has been renamed to, to reaction product from paraformaldehyde and 2-hydroxypropylamine, ratio 3:2 (in short RP 3:2). It has to be noted that studies submitted for evaluation referring to MBO as active substance are in reality describing the properties of RP 3:2.

Studies referring to MBO are (theoretically) assuming that RP 3:2 would have completely reacted to MBO. Further it can be said that hydrolysis of 1 mol MBO, by addition of 3 mol water, would result in 3 Mol formaldehyde and 2 mol 2-hydroxypropylamine (see Figure 1.1-1).

Therefore studies calculate the maximum amount of formaldhyde which can be released from MBO (correctly described as RP 3:2) in one of the following ways:

	Molar mass [g]	% refered to MBO(*)	% refered to MBO + 3 mol water
2 mol 2-hydroxypropylamine	150	80,6	62,5
3 mol Formaldehyde	90	48,4	37,5
Sum	240	129,0	100,0

(*) Molar mass of MBO = 186

1.2 Composition of the substance

The minimum degree of purity cannot be set for the UVCB substance. The active substance is identified by its source and the manufacturing process.

There are no additives in the substance as manufactured.

Current Annex VI entry: No current Annex VI entry.

1.2.1 Composition of test material

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: CONTRAMTM MB, GrotaMar71® and Grotan® OX.

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico - chemical properties

Endpoint	Method	Purity/Specification	Results	Reference
Melting point	EC method A.1	3,3'-methylenebis[5-methyloxazolidine]; Lot No.: 24773 Content of easy releasable formaldehyde: 43.52% w/w Content of total formaldehyde: 42.28% w/w Water content: 0.43% w/w	- 60.5°C (Freezing point:-29.5°C)	**Doc. III-A 3; Study A 3.1.1/01**
	EC method A.1	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 16.48% 2 hydroxypropylamine: 77.79%	<-35°C	**Doc. III-A 3; Study A 3.1.1/02**
Boiling point	EC method A.2	3,3'-methylenebis[5-methyloxazolidine]; Lot No.: 24773 Content of easy releasable formaldehyde: 43.52% w/w Content of total formaldehyde: 42.28% w/w Water content: 0.43% w/w	192.2°C	**Doc. III-A 3; Study A 3.1.2/01**
	EC method A.2	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	204.3°C	**Doc. III-A 3; Study A 3.1.2/02**
Relative density	EC method A.3	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	mean relative density: 1.05 (20°C)	**Doc. III-A 3; Study A 3.1.3/01**
	DIN 51757	CONTRAM™ MBO, Batch no.100496595	Density: 1.065 g/cm^3 at 20°C	**Doc. III-A 3; Study A 3.1.3/02**
Vapour pressure	EC method A.4 calculated	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	5.83 hPa (25°C), calculated from regression curve	**Doc. III-A 3; Study A 3.2/01**
	EC method A.4 calculated	3,3'-methylenebis[5-methyloxazolidine]; Lot No.: 24773 Content of easy separable formaldehyde: 43.52% w/w Content of total formaldehyde: 42.28% w/w	2 Pa (20°C); 2.8 Pa (25°C); 13.9 Pa (50°C); calculated The calculated vapour pressure vales are extrapolated.	**Doc. III-A 3; Study A 3.2/02**
	calculated with epi suite3.12	n.a.	0.014 hPa (25°C)	**Doc. III-A 3; Study A 3.2/03**
Henry´s Law Constant	Epi Suite 3.12 HENRYWI	n.a.	0.011 Pa x m^3/mol (calculated with EpiSuite 3.12)	**Doc. III-A 3; Study A 3.2/03**

	N v3.10			
Physical state	Visual inspection	n.a.	liquid	**Doc. III-A 3; A3.1.1/01 an d Study A3.1.1/02**
Colour	Visual inspection	n.a.	colourless to yellowish	**Doc. III-A 3; A3.1.1/01 an d Study A3.1.1/02**
Odour	Olfactory inspection	n.a.	amine like	**Doc. III-A 3; Company Statement**
Absorption spectra: UV/VIS	Spectralphotometric determination	CONTRAM™ MBO, Lot Number 30000334619	UV/VIS spectrum is consistent with the proposed structure of MBO. There are no absorption maxima above 290 nm.	**Doc. III-A 3; Study A 3.4/01 UV/VIS**
	Spectralphotometric determination	GrotaMar 71; Charge 1092500	UV/VIS spectrum is consistent with the proposed structure of MBO. There are no absorption maxima above 290 nm.	**Doc. III-A 3; Study A 3.4/02 UV/VIS**
Absorption spectra: IR	Spectralphotometric determination	CONTRAM™ MBO, Charge 100496595 Purity?	IR spectrum is consistent with the proposed structure of MBO.	**Doc. III-A 3; Study A 3.4/01 IR**
	Spectralphotometric determination	Grotan OX, Charge 1126714	IR spectrum is consistent with the proposed structure of MBO.	**Doc. III-A 3; Study A 3.4/02 IR**
Absorption spectra: NMR	1H-NMR 13C-NMR	CONTRAM™ MBO and GrotaMAR 71; Charge 1057472	1H- NMR spectrum and 13C- NMR spectrum is consistent with the proposed structure of MBO.	**Doc. III-A 3; Study A 3.4/01 NMR**
	1H-NMR 13C-NMR	MAR 71; Charge 1047946	1H- NMR spectrum and 13C- NMR spectrum is consistent with the proposed structure of MBO.	**Doc. III-A 3; Study A 3.4/02 NMR/MS**
Absorption spectra: MS	EI-MS	MAR 71; Charge 1047946	MS spectrum is consistent with the proposed structure of MBM.	**Doc. III-A 3; Study A 3.4/02 NMR/MS**
	VG Autospec sectorfield masspectrometer	CONTRAM™ MBO, Charge 100496595	MS spectrum is consistent with the proposed structure of MBM.	**Doc. III-A 3; Study A 3.4/03 MS**
Water solubility	OECD 105 (Flask method)	CONTRAM™ MBO; Lot.No.:24773 100 % Purity	Test substance is completely miscible at room temperature.	**Doc. III-A 3; Study A 3.5/01**
	EC method	GrotaMar 71; Batch-no.: 1024828	2800g/L (30°C; pH 9.77)	**Doc. III-A 3;**

	A.6 (Flask method)	Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%		**Study A 3.5/02**
Dissociation constant	Justification	n.a.	Test substance is hydrolysable, therefore determination of the pKa is not possible.	**Doc. III-A 3; Justification**
Solubility in organic solvents, including the effect of temperature on solubility	OECD 116	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46% - 49% 2 hydroxypropylamine: 77% - 79%	Highly soluble in standard fat (HB 307) at 37°C No saturation up to 7.12 g / 2 g standard fat.	**Doc. III-A 3; Study A 3.7/01**
	visual inspection for turbidity	CONTRAM™ MBO	Completely miscible in DMSO, ethanol, n-Octanol and acetone Partially soluble in toluene,cyclohexane (21-23 °C)	**Doc. III-A 3; Study A 3.7/02**
	Hach Method 8195	CONTRAM™ MBO	Solubility in n-heptane: 500 – 1000 mg/L (20.5°C)	**Doc. III-A 3; Study A 3.7/03**
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. Therefore, stability in organic solvents is not applicable.	**Doc. III-A 3; Justification**
Partition coefficient n-octanol/water (log Kow)	EC method A.8 (Shake flak method)	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	Log Pow: result: -0.043 (mean) n-octanol/water 1:1: -0.075 n-octanol/water 1:2: -0.210 n-octanol/water 2:1: 0.156	**Doc. III-A 3; Study A 3.9/01**
	EC method A.8 (Shake flak method)	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46% - 48% 2 hydroxypropylamine: 77% - 79%	Log Pow: result: -0.2253 (based on formaldehyde) result: -0.5517 (based on IPA)	**Doc. III-A 3; Study A 3.9/02**
	OECD 117	3,3'-methylenebis[5-methyloxazolidine]; Lot No.: 24773	Log Pow: 1.89 (30°C)	**Doc. III-A 3; Study A 3.9/03**
Thermal stability	DSC screening Test	Mar71; Batch-no.: 11021;1060748	According to the Differential Scanning Calorimetry (DSC) - Screening test, at an onset-temperature of 186°C	**Doc. III-A 3; Study A 3.10/01**

			exothermal degradation is expected.	
	Differential Scanning Calorimetry (DSC)	CONTRAM™ MBO Batch-no.: 100495595	An onset-temperature of 190°C exothermal degradation has been obtained. Substance can be safely handled up to the flashpoint (73°C).	**Doc. III-A 3; Study A 3.10/02**
Flammability	EC method A.12	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	GrotaMar 71 is non-flammable and non-hazardous.	**Doc. III-A 3; Study A 3.11/01**
	EC method A.15	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	No flammable gas was evolved Autoignition temperature: 237°C (766 mm Hg).	**Doc. III-A 3; Study A 3.11/02**
Flash-point	EC method A.9	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	73 °C	**Doc. III-A 3; Study A 3.12**
Surface tension	EC method A.5 (ring method)	Grotan® OX; Batch no. 1121890; specification; Reaction mixture; Content 90-100%	Test item has no surface active properties. Surface tension of aqueous solution at 20°C is σ=68.1 mN/m.	**Doc. III-A 3; Study A 3.13**
Viscosity	OECD 114	Grotan OX; Charge 1121890;	21 m Pa 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	OPPTS 830.6314 EPA 712-C-96-023	Grota MAR 71®; Batch no. 1024828 Reaction mixture, active ingredient: Formaldehyde: 46.9 w/w, 2-hydroxypropylamine 80.2%w/w	Test active substance has no oxidising properties.	**Doc. III-A 3; Study A 3.16**
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. Experience shows that these materials are suitable for storage and transport of the biocide	**Doc. III-A 3; Study A 3.17**

2 MANUFACTURE AND USES

2.1 Manufacture

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

2.2 Identified uses

Disinfectants and algaecides not intended for direct application to humans or animals, product type 2
In-can preservative, product type 6
Preservatives for liquid-cooling and processing systems, product type 11
Slimicides, product type 12
Metal-working fluid, product type 13

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 3-1: Summary table for relevant physico-chemical studies

Property	Method	Purity/Specification	Results	Reference
Thermal stability identity of relevant breakdown products	DSC screening Test	Mar71; Batch-no.: 11021;1060748	According to the Differential Scanning Calorimetry (DSC) - Screening test, at an onset-temperature of 186°C exothermal degradation is expected.	**Doc. III-A 3; Study A 3.10/01**
	Differential Scanning Calorimetry (DSC)	CONTRAM™ MBO Batch-no.: 100495595	An onset-temperature of 190°C exothermal degradation has been obtained. Substance can be safely handled up to the flashpoint (73°C).	**Doc. III-A 3; Study A 3.10/02**
Flammability, including autoflammability and identity of combustion products	EC method A.12	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	GrotaMar 71 is non-flammable and non-hazardous.	**Doc. III-A 3; Study A 3.11/01**
	EC method A.15	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	No flammable gas was evolved Autoignition temperature: 237°C (766 mm Hg).	**Doc. III-A 3; Study A 3.11/02**
Flash point	EC method A.9	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	73 °C	**Doc. III-A 3; Study A 3.12**
Explosive properties	Justification	n.a.	There is no structural alert for explosive properties.	**Doc. III-A 3; Justification**
Oxidizing properties	OPPTS 830.6314 EPA 712-C-96-023	Grota MAR 71®; Batch no. 1024828 Reaction mixture, active ingredient: Formaldehyde: 46.9 w/w, 2-hydroxypropylamine 80.2%w/w	Test active substance has no oxidising properties.	**Doc. III-A 3; Study A 3.16**
Reactivity towards container	Company Statement	n.a.	The biocidal product is packed and stored in LDPE containers	**Doc. III-A 3; Study A 3.17**

Property	Method	Purity/Specification	Results	Reference
material			or in steel barrels or containers coated with LDPE. Experience shows that these materials are suitable for storage and transport of the biocide	

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

Grotan® WS as well as CONTRAMTM 121 are complex reaction mixtures produced by reacting 2-hydroxypropylamine with paraformaldehyde (ratio 1:1; RP 1:1). The main component is α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) which is also one major by-product of the “reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)”. In aqueous medium the complex reaction mixture including HPT hydrolyses back to 2-hydroxypropylamine and formaldehyde.

Grotamar 71 and Contram MBO are complex reaction mixtures produced by reacting paraformaldehyde with 2-hydroxypropylamine (ratio 3:2, RP 3:2). The main component is 3,3'-methylene¬bis[5-methyloxazolidine] (MBO) and one of the by-products is α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT). In aqueous medium the complex reaction mixture including MBO hydrolyses to HPT and 2-hydroxypropylamine and formaldehyde.

To get a better understanding of the toxicity of the overall mixtures, data on both of the reaction products, RP 1:1 and RP 3:2, have been assessed within this document and the hydrolysis products have been assessed within the Appendix “Formaldehyde Core Dossier” and Appendix “2-Hydroxypropylamine”.

A comparison of the effects is given in this document at the end of each section in tabulated form.

The reaction mixture 2-hydroxypropylamine with paraformaldehyde (ratio 1:1, RP 1:1) contains about 28% releasable formaldehyde and the reaction mixture 2-hydroxypropylamine with paraformaldehyde (ratio 3:2, RP 3:2) contains about 45% releasable formaldehyde.

This means that for comparison of formaldehyde data with data from the releaser mixtures, the formaldehyde data may be multiplied by a factor of 3.6 for the mixture with 1:1 ratio and with 2.2 for the mixture with 3:2 ratio.

For comparing data from the RP 1:1 with data from the RP 3:2 a factor of 1.6 is suitable in case comparison shall be based on formaldehyde content of the two mixtures.

Table 4-1 Conversion factors for reaction products with FA

	1:1 mixture	3:2 mixture	FA
1:1 mixture →		0.62	0.28
3:2 mixture →	1.6		0.45
FA →	3.6	2.2	

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information – RP 1:1 and RP 3:2

Data on toxicokinetics and metabolism cannot be obtained for a complex reaction mixture like the RP 1:1 and RP 3:2 discussed here. Moreover, data on toxicokinetics and metabolism of HPT or MBO as single compounds and main constituents cannot be obtained, as both are in a complex equilibrium with the reacting compounds and hydrolysis products in aqueous solutions.

Data on the hydrolysis product 2-hydroxypropylamine are not available. Data on formaldehyde, which is considered as the toxicologically most important constituent of the mixture (see appendix and tables in the following sections), are given below.

4.1.2 Non-human information – component of RP 1:1, RP 3:2 and hydrolysis product: formaldehyde

Table 4.1-1 Toxicokinetics and metabolism of formaldehyde

Endpoint	Formaldehyde (for details see Appendix Formaldehyde Core Dossier)		
	Dermal	Inhalation	Oral
Absorption	100 % uptake (based on ^{14}C in excreta, organs and carcass, and on in vitro data on human skin), systemic bioavailability low (first-pass metabolism)	100 % uptake (based on ^{14}C) (rodents/primates at rest: ~ 90 and 70 % in nasal passages, man/oronasal breathing: up to ~ 45 % tracheo-bronchially), systemic bioavailability below 10 % (first-pass metabolism)	100 % uptake, rapid (based on ^{14}C in exhaled air, urine and carcass), systemic bioavailability low (first-pass metabolism)
Distribution	systemic bioavailability low ^{14}C label widely distributed (introduction into C1-pool)		
Metabolism	1) Reaction with GSH followed by enzymatic conversion to formate and utilisation for C1-transfer or oxidation to CO2 2) Direct enzymatic conversion to formate and utilisation for C1-transfer or oxidation to CO2 3) Reaction with THF followed by conversion to 5-methyl or 5-formyl THF and utilisation for C1-transfer, or transformation to 10-formyl THF and release of formate or oxidation to CO2 4) Adduct formation with cysteine, urea, proteins and nucleic acids Pronounced first-pass metabolism at site of entry		
Toxicologically significant metabolite	Toxicity of metabolites not assessed separately Urine: formate, hydroxymethylurea		
Rate and extent of excretion	Metabolic elimination, high, but variable rate and extent of metabolite excretion (based on ^{14}C) mainly with air and urine (initial plasma t1/2 12 h, terminal t1/2 50 h, 10-40 % ^{14}C residues after 3-4 d)		

4.1.3 Human information

No data available for RP 1:1 and RP 3:2. For the hydrolysis product formaldehyde please see chapter 4.1.2 above.

4.1.4 Summary and discussion on toxicokinetics

No informative data can be generated for the complex reaction mixtures RP 1:1 and RP 3:2.However it can be considered that RP 1:1 and RP 3:2 hydrolyze quickly to formaldehyde and 2-hydroxypropylamine with contact to biological tissues and with dilution in aqueus media.

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.

No data are available for 2-hydroxypropylamine, but this hydrolysis product is considered of very minor toxicological relevance.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity – RP 1:1

Table 4.2-1 Summary of acute toxicity data of RP 1:1 in rats

Route	Method Guideline	Species Strain Sex no/group	dose levels	identity as given in study report	Value LD50	Remarks	Reference
Oral	LD_{50} study OECD 401	Rat Wistar 5 m & 5 f	0, 900, 1350, 2025 mg/kg bw; 0, 9, 13.5, 20.25% in distilled water	Grotan WS Batch 1025145 FA 27.9%	m & f combined: LD_{50} = 960 mg/kg bw	Local effects in the gastro-intestinal tract	Schülke & Mayr (2000), DocIIIA6.1.1
Dermal	LD_{50} study OECD 402	Rat Wistar 5 m & 5 f	Limit test 2000 mg/kg bw undiluted test substance	Contram 121 Batch 24774	LD_{50} > 2000 mg/kg bw in f and m (1 f died at day 4)	Mostly local corrosive effects in survivors	Becker Chemie (2002), DocIIIA6.1.2/01
Dermal	LD_{50} study OECD 402	Rat Wistar 5 m & 5 f	Limit test 2000 mg/kg bw undiluted test substance	Grotan WS Batch 1025145 FA 27.9%	LD_{50} > 2000 mg/kg bw in f and m (no mortality)	Incomplete data on local effects.	Schülke & Mayr (2000), DocIIIA6.1.2/02

f: females; m: males

The acute toxicity after oral and dermal exposure has been investigated in valid studies on experimental animals. The oral LD_{50} in rats is 960 mg/kg bw. Primarily local effects in the gastro-intestinal tract were observed (**cf. DocIIIA6.1.1**). The dermal LD50 in rats is higher than 2000 mg/kg bw. Local corrosive effects were noted, which were not reversible within the post exposure observation period (**cf. DocIIIA6.1.2/01**).

4.2.1.2 Acute toxicity – RP 3:2

Table 4.2-2 Acute oral and dermal toxicity of RP 3:2 in rats

Route	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	identity as given in study report	Value LD50/LC50	Remarks	Reference
Oral	Comparable to OECD 401	Rat Sprague-Dawley 10 m & 10 f	0.5, 0.64, 0.79, 1.00, 1.26 ml/kg bw in water (0.9% NaCl solution)	FO-IVP 1262, MK-ÄI2P	ca. 750 mg/kg bw for males and females	Concentration at LD50 about 8%	Schülke & Mayr (1977); DocIII A6.1.1/01
Oral	Comparable to OECD 401	Rat Sprague-Dawley 5 m & 5 f	270, 530, 670, 850, 1060, 1340 mg/kg bw in water (0.9% NaCl solution)	N,N-Methylen-bis(5-methyl oxazolidin)	LD50 for males 900 and for females 920 mg/kg bw	Concentration at LD50 about 10%	Schülke & Mayr (1979); DocIII A6.1.1/02
Oral	OECD 423 GLP	Rat Sprague-Dawley 3 m & 3 f	2000 & 200 mg/kg bw in corn oil (acute toxic class method)	Contram MBO total FA 42,28%	LD50 = 630 mg/kg bw for males and females Mortality: 100% with 2000 mg/kg bw (neat); no effects with 200 mg/kg bw (~10% solution)		Bode Chemie (2002); DocIII A6.1.1/03
Dermal	Comparable to OECD 402	Rat Sprague-Dawley 5 m & 5 f	2.52, 3.18, 4.00, 5.04, 6.35 ml/kg bw undiluted substance	FO-IVP 1262, MK-ÄI2P	LD50 ca. 6000 mg/kg bw for males and females	LD50 value clearly above others Only skin reddening with ≥ 5 ml/kg bw (questionable dilution)	Schülke & Mayr (1977); DocIII A6.1.2/01
Dermal	OECD 402 GLP	Rat Wistar 5 m & 5 f	0, 1000, 1350, 1823 mg/kg bw undiluted substance,	GrotaMAR 71 Batch 1024828 FA 46.9% HPA 80,2%	LD50 = 1400 mg/kg bw for males and females combined mortality: 10/50/80% with increasing dose	≥ 1000mg/kg bw: Epidermal thickening/ erythema, scab	Schülke & Mayr (2000); DocIII A6.1.2/02

Dermal	OECD 402 GLP	Rat Sprague-Dawley 5 m & 5 f	250, 750, and 2000 mg/kg bw ~ 13%, 40% in corn oil and undiluted	Contram MBO Charge 24773 FA 42.28%	LD50 = 790 mg/kg bw for males and females combined; mortality: 1/10 animals in 750 mg/kg bw, 10/10 animals in 2000 mg/kg bw	With 2000 mg/kg bw erythema and oedema (all) and necrosis (2 animals) in high dose	Bode Chemie (2002); DocIII A6.1.2/03

The acute toxicity after oral and dermal exposure has been investigated in valid studies on rats. The oral LD50 ranged from 630 to 920 mg/k bw. Clinical signs observed in rats after oral application were sedation, ataxia and dyspnea 5-10 minutes after application followed by coma and death. Pathology revealed no treatment related effects (Schülke & Mayr, 1977, cf. DocIIIA6.1.1/01). Similar results were reported in two further oral studies (surprisingly no local effects detected cf. DocIIIA6.1.1/02-3).

The dermal LD50 in rats ranged from 760 to 6000 mg/kg bw. Lethargy, local erythema, abdominal breathing, nostril discharge and piloerection on day 1 and 2 were reported after acute dermal exposure and at higher dose levels additionally tremor and gasping. The local skin effects (necrosis) were not reversible within 14 days (Schülke & Mayr, 2000, cf. DocIII A6.1.2/02). Similar results were presented by Bode Chemie including ataxia and dyspnoea intermediate dose (2002, cf. DocIII A6.1.2/03). In both studies no treatment-related findings were detected at necropsy except local effects (scab formation).

Clinical signs after application and the dose-effect-level suggested similar absorption pattern of the test substance after oral and dermal exposure (presuming that effects are not exclusively secondary to local necrosis after dermal application).

4.2.1.3 Comparison of RP 1:1, RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.2-3 Comparison of acute toxicity data of the RP 1:1, RP 3:2 and its components

Endpoint	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde (FA)**
Acute oral toxicity	Rat LD_{50} = 960 mg/kg bw (as ~10% aqueous solution) congestion of stomach, intestine and lungs, mottling in liver	Rat LD_{50} = 630 mg/kg bw (as ~10% aqueous solution) mortality: 100% with 2000 mg/kg bw (neat); no effects with 200 mg/kg bw (~10% solution) no findings at necropsy	Rat LD50 = 640 mg/kg bw (as ~4% aqueous solution) local effects not reported but expected from repeated dose toxicity studies
Acute dermal toxicity	Rat LD_{50} > 2000 mg/kg bw (undiluted) corrosive effects	Rat LD_{50} = 790 mg/kg bw mortality: 10% in 750 mg/kg bw (ca 40% a.s. in corn oil), 100% in 2000 mg/kg bw (neat a.s.) corrosive effects with undiluted substance	Rabbit LD50 = 270 mg/kg bw corrosive
Acute inhalation toxicity	No data available	No data available	LC50(4h) = 0.6 mg/L (rat)

4.2.2 Human information for RP 1:1 and RP 3:2

Not available.

4.2.3 Summary and discussion of acute toxicity

The acute toxicity testing results are not straight forward to compare since lethality expectedly depends on the dose and the concentration of the substances. Furthermore the newer acute toxicity tests do not allow estimating an exact LD50 but just the estimation of a toxicity category or no classification in case of the limit tests.

The available data as summarised above would support classification and labelling according to the Regulation (EC) No 1272/2008 as follows:

4.2.4 Comparison with criteria

For Formaldehyde (harmonised classification)

Acute oral toxicity: Category 3*, Toxic if swallowed, H301

Acute dermal toxicity: Category 3*, Toxic in contact with skin, H311

Acute inhalation toxicity: Category 3*, Fatal if inhaled, H331

For the RP 1:1 and RP 3:2:

Acute oral toxicity: Category 4, Harmful if swallowed, H302

Acute dermal toxicity: Category 4, Harmful in contact with skin, H311 for the 3:2 mixture (3 study results available: 6000, 1400, < 2000 mg/kg bw for undiluted substance), but not for the 1:1 mixture (2 study results available, both LD50 > 2000 mg/kg bw for undiluted substance)

Acute inhalation toxicity: Category 4, Harmful if inhaled, H332 (based on read across from formaldehyde vapour to releaser mist with 28% FA content)

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. Therefore we propose no acute toxicity classification for the 3:2 and the 1:1 reaction product

4.2.5 Conclusions on classification and labelling

No classification is required.

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

RP 1:1 and RP 3:2 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.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Human information for RP 1:1 and RP 3:2

Not data are available.

4.4.1.2 Non-human information for RP 1:1

Table 4.4-1 Skin irritation of the RP 1:1

Species	**Method**	**identity as given in study report**	**Score 1h, 24h, 48h, 72h / average score 24,48,72 h after patch removal**		**Rever-sibility**	**Result / remarks**	**Reference**
			Erythema	Edema	yes/no		
Rabbit n= 3	OECD 404; undiluted test substance GLP	Contram 121 Batch 24774	2.6, 2.6, 2.3 / 2.5	2.3, 2.0, 1.7 / 2.0	No	Evidence for damage of deeper skin layers; strong irritant to corrosive properties	**Becker Chemie (2002); DocIIIA6.1.4/01**
Rabbit n= 3	OECD 404; undiluted test substance	Grotan WS Batch 1025145 FA 27.9%	1.0, 1.3, 1.7 / 1.33	1.0, 1.3, 1.7 / 1.33	Yes	Eschar formation at day 7 (no effects at day 14);	**Schülke & Mayr (2000); DocIIIA6.1.4/02**

In both studies available on skin irritation the results indicated tissue damage of deeper skin layers after dermal exposure to the undiluted test substance. However, there is some delay in effects. Especially in the 2nd study (cf. DocIIIA6.1.4/02) lesions of deeper skin layers were obvious later than 72 h after patch removal. In studies on sensitization (cf. DocIIIA6.1.5/01) irritant effects were found in guinea pigs at a concentration of 10% in Alembicol D but no irritation at a concentration of 5% (occlusive dressing for 24 h; n=10).

The overall results suggested strong irritant to corrosive properties of the undiluted test substance and irritant effects at a concentration of 10%. No local effects were detected at a concentration of 5%.

4.4.1.3 Non-human information for RP 3:2

Table 4.4-2 Skin irritation of the RP 3:2

Species	Method	identity as given in study report	Score 1h, 24h, 48h, 72h / average score 24,48,72 h after patch removal		Reversi-bility	Remarks/results	Reference
			Erythema	Edema	yes/no		
Rabbit	Comparable with OECD 404 but restrictions, 24 h exposure, occlusive	N,N-Methylen-bis (5-methyl oxazolidin)	1.8, 2.0, 1.3, 1.0 / 1.4	Scoring not reported according to OECD standards	yes	Irritant with 24h exposure; slight irritation with 25% aqueous solution test substance not applied directly to the skin	Schülke & Mayr (1976); DocIII A6.1.4/01
Rabbit	Comparable with OECD 404 but restrictions 24 h exposure	Grotan OX Ch B 9190	3.8 (1 h), 3.8 (48 h)	3.8 (1 h), 3.8 (48 h)	No data	Corrosive with 24h exposure, last reading at 48 h	**Schülke & Mayr (1979); DocIII A6.1.4/02**
Rabbit	OECD 404 4 h exposure semi-occlusive	3,3´-Methylen bisoxolidin Batch 24773	2.8, 2.5, 3.2, 4.0 / 3.2	4.0, 2.0, 2.0, 2.0 / 2	No	Corrosive	**Bode Chemie (2002); DocIII A6.1.4/03**

In an older study (Schülke & Mayr 1976, cf. DocIIIA6.1.4/01) reversible irritant effects were reported in rabbits exposed for 24 h (4 h recommended) to the neat test substance. The results of this study are in contrast to the findings of corrosivity in two other studies, eventually because in the study from1976 the test substance was not applied directly to the skin. Schülke & Mayr (1979, cf. DocIIIA6.1.4/02) also exposed rabbits for 24 h. There was evidence that the test substance causes burns after this exposure period. No data were available on the reversibility of these effects (limited documentation) but it can be concluded from this study that the test substance has corrosive properties. In a 3rd study conducted according to OECD guideline 404 (Bode Chemie 2002, cf. DocIII A6.1.4/03) 4 h dermal exposure to 0.5 ml test substance resulted in irreversible destruction of skin tissue.

Threshold concentration for acute skin irritation was determined in preliminary investigations of a study on skin sensitization in guinea pigs (GPMT): no effects were detected at 1% but slight irritation at 5% in aqueous solutions and slight to moderate irritation at 10% (1 out of 6 animals with necrotic patch) (cf. DocIII A6.1.5/01). These acute threshold concentrations were confirmed in a 2nd GPMT (cf. DocIII A6.1.5/03).

4.4.1.4 Comparison of RP 1:1, RP 3:2 with its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.4-3 Comparison of the active substance and its components

Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)	Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)	Formaldehyde	2-Hydroxy-propylamine
Causes burns	Causes burns	Causes burns Corrosive properties related to reaction at the site of contact	Causes burns Corrosive properties related to high pH value

4.4.1.5 Summary and discussion of skin irritation

Several studies for skin irritation are available for the RP 1:1 as well as the RP 3:2. The results are not fully reproducible with regard to scores and reversibility. However limited reproducibility is well known for these in vivo test methods.

However more weight was given to the newer studies and also the corrosive properties of the hydrolysis product formaldehyde was considered.

4.4.1.6 Comparison with criteria

Giving more weight to the newer studies and considering also the corrosive properties of the hydrolysis product formaldehyde irreversible skin damage was apparent for RP 1:1 as well as RP 3:2. This is supportive for classification in skin corrosion category 1.

Only in the study from 2002 with RP 1:1 in addition to the 4 hours exposure also 3 minutes and 1 hour exposure times were tested. However the results section mentions only “well defined erythema” 4 hours post exposure for these two shorter exposure times. For all other studies the application time was just 4 hours. Therefore no differentiation between category 1A, B or C is possible.

4.4.1.7 Conclusions on classification and labelling

It is concluded that RP 1:1as well as RP 3:2 should be classified as Skin Corrosive Category 1, H314 - Causes severe skin burns and eye damage.

4.4.2 Eye irritation

Due to the skin corrosive effects no in vivo eye irritation studies must be carried out. The following studies were not required by the RMS, but nevertheless provided by the applicant. Consequently they are summarized here

4.4.2.1 Non-human information for RP 1:1

Table 4.4-4 Eye irritation of RP 1:1 in rabbits

Species	Method	a.s. source	Average Score 24, 48, 72 h after instillation				Reversibility	Remarks/ results	Reference
			Cornea	Iris	Chemosis Conjunctiva	Redness Conjunctiva	Yes/No		
Rabbit	OECD 405	Grotan WS Batch 1025145 FA 26.4-28%	0.67	0	1.7	2	No		Schülke & Mayr, 2000; **cf. DocIIIA6.1.4/03**

In an acute eye irritation study in 3 rabbits according to OECD guideline 405 (Schülke & Mayr, 2000; **cf. DocIIIA6.1.4/03**) the application of 0.1 ml of the undiluted test substance (Grotan WS) resulted in only moderate erythema and oedema but which were not completely reversible after 21 days. However, long-lasting lesions of the cornea have been demonstrated which were not reversible. It was concluded that the test substance was corrosive to the eyes.

4.4.2.2 Non-human information for RP 3:2

Table 4.4-5 Eye irritation of RP 3:2 in rabbits

Species	Method	identity as given in study report	Average Score 1, 24, 48, 72 h after instillation				Reversibility	Remarks/ results	Reference
			Cornea	Iris	Chemosis Conjunctiva	Redness Conjunctiva	Yes/No		
Rabbit	Comparable to OECD 405	Grotan OX Ch B 9190	2.3 (24 h)	2.0 (24 h)	4.0 (24 h)	3.0 (24 h)	Rabbits sacrificed	Serious damage by the undiluted test substance; similar results with washing eyes after 4 s exposure; 0.2% in water not irritant	**Schülke & Mayr (1979) DocIII A6.1.4/04**
Rabbit	No guideline	Abt. FO-IL VP 1262	-	-	0, 1.8, 1.6, 0.2	1.0, 2.0, 1.2, 0.4	No (after 7 d)	Not valid, additional information only	Gray Products (1978) DocIII A6.1.4/05

Irreversible severe effects were observed in the more valid Guideline study from 1979.

4.4.2.3 Human information for RP 1:1 and RP 3:2

No human data available.

4.4.2.4 Comparison of RP 1:1, RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.4-6 Comparison of the RP 1:1, RP 3:2 and its components

Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Causes burns	Causes burns	Causes burns

4.4.2.5 Summary and discussion of eye irritation

Due to the skin corrosive effects no in vivo eye irritation studies must be carried out. The above summarized studies were not required by the RMS, but nevertheless provided by the applicant. The studies support the available knowledge of severe irreversible local effects.

4.4.2.6 Comparison with criteria

The observed severe, irreversible eye damage would support the classification for eye damage cat 1.

4.4.2.7 Conclusions on classification and labelling

RP 1:1 and RP 3:2 should be classified for skin corrosion Cat 1, no further classification for local eye effects necessary.

4.5 Corrosivity

See chapter 4.4

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information for RP 1:1

Table 4.6-1 Sensitization of RP 1:1 in experimental animals

Species	Method	identity as given in study report	Number of animals sensitized/total number of animals	Result / Remarks	Reference
Guinea pig	Guinea pig maximisation test (GPMT) according to OECD406 GLP Intradermal induction 1% (v/v), topical induction 25%; topical challenge with 10, 5, 2.5, 1%.	OS157338	Rechallenge concentration 1%: 18/20 (24, 48, 72 h after challenge) 2.5%: 19/20 no effects in controls	1st challenge concentration of 10% resulted in slight irritation in controls, but moderate to severe irritation in test animals. Conclusion rechallenge: high potency skin sensitisation: with intradermal induction dose of 1% more than 60% response	**Lubrizol Corporation (2001); DocIIIA6.1.5/01**
Guinea pig	Guinea pig maximisation test (GPMT) according to OECD406 Intradermal induction 1% (v/v) in distilled water, topical induction undiluted; topical challenge undiluted	Grotan WS Batch 1025145 FA 27.9%	Challenge with **undiluted** test substance: 8/20; no effects in 10 controls	Authors conclusion: sensitizing; not reliable study (K.-score 3) since unclear study report and contradiction to strong irritant to corrosive properties of undiluted active substance shown in irritation tests.	Schülke & Mayr (2001); DocIIIA6.1.5/02

For this endpoint one reliable study is available (see table above). In a Guinea pig maximisation test (GPMT, cf. DocIIIA6.1.5/01) evidence for skin sensitisation has been shown. An intradermal and epidermal induction dose of 1% and 25% in Alembicol D, respectively was chosen in this test. The concentration of 10% used for challenge was irritant in controls, however sensitizing but no irritant effects were found after challenge with 5% and rechallenge with 2.5 und 1% solutions of the test substance. After challenge with 1% solution score 1-2 (one animal score 3) was detected in 18/20 animals and no skin reaction in 2/20. Considering the intradermal induction dose of 1% and more than 60% positive animals after challenge and re-challenge the active substance is considered as high potency skin sensitizer (GHS Cat 1A). The second GPMT (DocIIIA6.1.5/02) also applied 1% intradermal induction, but undiluted topical induction and undiluted topical challenge and resulted in maximally 40% positive animals. However the study was considered as not reliable due to unclear study report and contradiction to strong irritant to corrosive properties of undiluted active substance shown in irritation tests.

4.6.1.2 Non-human information for RP 3:2

Table 4.6-2 Sensitization of RP 3:2 in guinea pigs

Species	Method	identity as given in study report	Number of animals sensitized/total number of animals	Result / remarks	Reference
Guinea pigs	Guinea pig maximisation test (GPMT) according to OECD 406 GLP Intradermal induction 0.01%, topical induction 10%; topical challenge 1 and 5% (v/v) in Alembicol D	OS157339	Challenge concentration 1%: 2/20 (24 h after challenge); 1/19 (after 48 h); 0/20 (after 72 h)	Not sensitizing, but concentration for intradermal induction not sufficient	Lubrizol Corporation (2001); DocIII A6.1.5/01
Guinea pigs	GPMT according to OECD 406 GLP Intradermal induction 5% in distilled water, topical induction undiluted; topical challenge 75% in distilled water	GrotaMAR 71 FA 46-48% HPA 77-19%	Challenge concentration 75%: 19/20 (24 h after challenge); 18/20 (after 48 h)	skin sensitizer with high intradermal induction dose of 5% more than 90% response	**Schülke & Mayr (2001); DocIII A6.1.5/02**
Guinea pigs	GPMT, comparable to OECD 406 Intradermal induction 0.5% in water, topical induction 10% in water; topical challenge 1, 0.5, 0.1% in petrolatum	Grotan OX FA ~4% from 10% aqueous solution	Challenge concentration 1% in petrolatum: 12/20; 0.5%: 7/20; 0.1%: 2/20 (all 48 h after challenge)	High potency skin sensitizer: with intradermal induction dose of 0.5% ≥ 60% response	**Anderson et al. (1984); DocIII A6.1.5/03**

In the Guinea pig maximisation test (GPMT) presented by Lubrizol Corporation (2001, cf. DocIIIA6.1.5/01) no evidence of skin sensitisation animals was detected. However, the concentration of the test substance was not sufficient for induction (only 4/20 animals showed reactions other than the control values) limiting the reliability of this study.

In a 2nd GPMT conducted according to OECD guideline 406 (Schülke & Mayr, 2001, cf. DocIIIA6.1.5/02) it has been shown that the test substance is sensitizing. This study has some limitations: 1) no documentation of skin effects after induction (but results of the pilot study are available and positive results obtained in the main study); 2) for challenge 75% test substance in distilled water was used which should normally result in irritant effects (see Section 3.3, skin irritation) and there is some contradiction between the results in this pilot study and the OECD guideline study 404 on skin irritation, however, the positive outcome of this study was validated by negative results in controls. In conclusion, the limitations of the study are not sufficient to disprove the outcome of this study.

Another GMPT study was reported from Anderson et al. (1984, cf. DocIII A6.1.5/03). A moderate irritant concentration was applied for intradermal (0.5% in water) and topical (10%) induction as well as non-irritant concentrations (0.1, 0.5, or 1.0%) for challenge. A positive reaction in 60% of exposed animals was detected indicating high potency skin sensitizing activity (GHS Cat 1A). Ambiguous results were obtained at a challenge concentration of 0.1% (2/20 positive, control 1/19).

4.6.1.3 Human information for RP 1:1

No human data available.

4.6.1.4 Human information for RP 3:2

Numerous formaldehyde releasers were tested in the study published by Geier et al. (1997, cf. DocIIIA6.12/01). RP 3:2 has been shown to induce the highest frequency of contact allergy. In a group of 1786 patients 55 patients (or 3.1%) showed a positive reaction after exposure to the active substance. In this study 1406 patients were tested with Grotan®OX and additionally with formaldehyde. 46 out of 1406 showed a positive reaction with Grotan®OX and in 13 out of these 46 patients a positive reaction was also observed with formaldehyde. The author suggested -as most simple and plausible hypothesis- that the formaldehyde releaser might induce sensitizing effects primarily via the whole reaction mixture and not only from released formaldehyde.

Further evidence for sensitizing activity in humans is presented by Schnuch et al. (1998, cf. DocIIIA6.12/02) and Brinkmeier et al. (2002, cf. DocIIIA6.12/03; small number of patients) reporting similar results.

Overall conclusion: There is evidence for skin sensitizing properties of RP 3:2 in humans and experimental animals.

4.6.1.5 Comparison of RP 1:1, RP 3:2 with its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.6-3. Comparison of the RP 1:1, RP 3:2 with its components

Endpoint	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Sensitization in experimental animals	Sensitizing	Sensitizing	Sensitizing
Sensitization in human	No data	Sensitizing	Sensitizing

4.6.1.6 Summary and discussion of skin sensitisation

The available GPMTs for RP 1:1 and RP 3:2 are limited in their reproducibility. However limited reproducibility is common in such animal experiments and differences in the identity of these complex reaction mixtures may contribute to this. However the studies considered as valid support strong potency skin sensitizing properties for RP 1:1 and RP 3:2. In addition human skin sensitization to RP 3:2 is reported.

The hydrolysis product formaldehyde is a well-known human skin sensitizer. Also mechanistic considerations of total releasable amount of formaldehyde upon contact with biological media support the conclusion.

4.6.1.7 Comparison with criteria

Considering the GPMT for RP 1:1, the intradermal induction dose of 1% and more than 60% positive animals after challenge and re-challenge, the RP 1:1 can be considered as high potency skin sensitizer (Cat 1A).

Considering the GPMT for RP 3:2, the intradermal induction dose of 0.5% and the 60% positive animals after challenge with a 1% solution, the RP 3:2 can be considered as high potency skin sensitizer (Cat 1A).

4.6.1.8 Conclusions on classification and labelling

Classification is proposed for skin sensitization Cat 1A, H317 – May cause an allergic skin reaction.

4.6.2 Respiratory sensitisation

No data are available.

4.7 Repeated dose toxicity

4.7.1 Non-human information RP 1:1

Table 4.7-1 Repeated dose toxicity of RP 1:1 in rats

Route	**duration of study; guideline**	**Species Strain Sex no/group**	**dose levels frequency of application**	**identity as given in study report**	**Results / Remarks**	**LO(A)EL**	**NO(A)EL**	**Reference**
Oral (gav-age)	14 days; GLP	Rat Wistar 5 m & 5 f	0, 50, 100, 200 mg/kg bw; = 2, 4, 8% in peanut oil; once daily, 7 days/week	Contram 121 batch 24774	Body weight and food consumption ↓ at 200 mg/kg bw. Dose-range finding for 90d study;	200 mg/kg bw	100 mg/kg bw	Becker Chemie (2002); DocIIIA6.3.1/01
Oral (gav-age)	14 days; GLP	Rat Wistar 5 m & 5 f	0, 100, 250, 400 mg/kg bw = 1, 2.5, 4% in water; once daily, 7 days/week	Grotan WS batch 1025145 FA 26.4-28% HPA 68-71%	400 mg/kg bw: clinical symptoms and slightly reduced food consumption & body weight in m&f. 250 mg/kg bw: reduced kidney weight. Dose-range finding for 90d study	250 mg/kg bw	100 mg/kg bw	Schülke & Mayr (2002); DocIIIA6.3.1/02
Oral (gav-age)	90 Days; OECD 408 GLP	Rat Wistar 10 m & 10 f	0, 12, 30, 80, 150 mg/kg bw = 0, 0.48, 1.2, 3.2 or	Contram 121 batch 24774	≥ 80 mg/kg bw: clinical signs (breathing sounds), mortality,	80 mg/kg bw	30 mg/kg bw	**Lubrizol Deutschland GmbH (2002); DocIIIA6.4.1/01**

			6 % in peanut oil; once daily, 7days per week		lesions of larynx and pharynx; 150 mg/kg bw: lesions of oesophagus in f			
Oral (gav-age)	90 Days; OECD 408 GLP	Rat Wistar 10 m & 10 f	0, 40, 100, and 250 mg/kg bw; once daily, 7 days per week	Grotan WS batch 1025145 FA 26.4-28% HPA 68-71%	Invalid study Authors conclusion on NOAEL and LOAEL not comprehensible.	100 mg/kg bw/day (authors conclu-sion)	40 mg/kg bw/day (authors conclu-sion)	Schülke & Mayr (2002); DocIIIA6.4.1/02

In a 90-day gavage study according to OECD guideline 408 (cf. DocIIIA6.4.1/01) rats received 0, 12, 30, 80, 150 mg/kg bw/day corresponding to a concentration of 0, 0.48, 1.2, 3.2 or 6% in corn oil (application volume 2.5 ml/kg bw). No treatment related effects were noted at a dose of 30 mg/kg bw/day (1.2%). Dose levels of 80 mg/kg bw/day (3.2%) and above resulted in clinical symptoms like breathing sound and treatment-related mortality. In rats which died during the exposure period histopathological effects in larynx and pharynx (only high dose) were found. In 3 out of 9 females of the high dose group inflammation of the oesophagus was detected. In this 90-day gavage study the NOAEL was 30 mg/kg bw/day. The second 90 day oral gavage study (cf. DocIIIA6.4.1/02) is not considered valid due to the fact that the MTD was not clearly reached, no local GI effects were reported which is in disagreement with all other study results, some inflammatory responses are unclear and eventually due to mycoplasmal pneumonia and no historical control data were submitted.

4.7.2 Non-human information – RP 3:2

Table 4.7-2 Repeated dose toxicity of RP 3:2 in rats

Route	**duration of study; guide-line**	**Species Strain Sex no/group**	**dose levels frequency of application**	**identity as given in study report**	**Results / Remarks**	**LO(A)EL**	**NO(A)EL**	**Reference**
Oral gavage	14 days; no	Rat Wistar 5 m & 5 f	0, 72, 180, 450 mg/kg bw, in water, no data on concentration; once daily, 7 d per week in water	Grotamar 71 FA 46-48% HPA 77-79%	Clinical effects and mortality in the high dose group / Dose range finding study (limited parameters investigated)	-	-	Schülke & Mayr (2001); DocIIIA6.3.1/01
Oral gavage	28 days; no	Rat Sprague-Dawley 5 m & 5 f	0, 100, 300, 900 mg/kg bw = 0, 2, 6, 18% in corn oil; once daily, 7 d/ week	Contram MBO FA 42.28%	High dose: high mortality (termination day 6); mid dose: local effects in the stomach, mortality; low dose: body weight and food consumption↓; dose range finding study	100 mg/kg bw/day	-	Bode Chemie (2002); DocIIIA6.3.1/02

Oral gavage	92 days; OECD 408	Rat Wistar 10 m & 10 f	0, 30, 72, 180 mg/kg bw = 0.3, 0.72, 1.8% in water; once daily, 7 d /week	Grotamar 71 FA 46-48% HPA 77-79%	Slight effects on body weight and clinical chemistry parameters at the high dose level. Limited validity.	180 mg/kg bw/day question-able	72 mg/kg bw/day question-able	Schülke & Mayr (2001); DocIIIA6.4.1/01
Oral gavage	90 days; OECD 408	Rat Sprague-Dawley 10 m & 10 f	0, 20, 60, 180/120 mg/kg bw = 0.4, 1.2, 2.4% in corn oil once daily, 7 d /week	Contram MBO FA 42.28%	At ≥ 60 mg/kg bw local effects in the stomach; other effects secondary to this lesion (granulocytes ↑, lymphocytes ↓, only 180/120 mg/kg bw: pupil size ↓)	60 mg/kg bw/day	20 mg/kg bw/day	**Bode Chemie (2002); DocIIIA6.4.1/02**

No data are available on effects of the active substance after repeated dermal and inhalation exposure.

In a subchronic gavage study according to OECD guideline 408 (Schülke & Mayr, 2001, cf. DocIIIA6.4.1/01) slight effects on body weight gain and alterations in clinical chemistry in males of the high dose group have been detected. These data suggested a LOAEL of 180 mg/kg bw/day. However, concerning clinical chemistry parameters no historical control data of this laboratory were given. The toxicological relevance of other effects was questionable. No local effects in the stomach were found although such effects are expected. These data suggest that the MTD was not reached in this study. Furthermore, pulmonary infection due to Mycoplasma spec. has been detected in all groups including controls. Altogether, this study has limitations.

In a 2nd subchronic gavage study (OECD guideline 408; Bode Chemie, 2002, cf. DocIIIA6.4.1/02) the test substance induced local effects in the stomach at a dose level of ≥ 60 mg/kg bw. Other effects at the mid and high dose level (% of granulocyes increased, % of lymphocytes decreased), are considered to be a consequence of this chronic ulcerative gastritis & peritonitis. The toxicological relevance of the reduced pupil size detected in males and females of the high dose group is not clear. The dose levels of 0, 20, 60, 180/120 mg/kg bw/day correspond to a concentration of 0, 0.4, 1.2, 3.6/2.4% in corn oil. Effects in the stomach were detected at a concentration of 1.2%.

In a developmental toxicity study (according to OECD guideline 414; see Section 4.8.1) rabbits were gavaged with 0, 5, 45, 90, 135 mg/kg bw/day corresponding to a concentration of 0, 0.25, 2.25, 4.5, 6.75% in corn oil. A dose of 135 mg/kg bw/day resulted in severe maternal toxicity like a decrease in body weight, increased mortality and abortions. Necropsy revealed local lesions in the stomach of dams and an increased incidence in dilatation of the renal pelvis. There is some evidence that at least an increased incidence of lesions in the stomach occurred also at 45 mg/kg bw. Thus, effects in the stomach of rabbits were detected at a concentration of 2.25% (LOAEC).

The implementation of a subchronic oral study in a 2nd species is scientifically unjustified because mainly local concentration dependent effects are expected with the active substance which have been sufficiently demonstrated. Furthermore, the implementation of a sub-acute or sub-chronic dermal toxicity study in rats is scientifically unjustified because of the corrosive properties of the active substance.

Chronic studies are available for formaldehyde and these studies indicated local effects at the site of contact.

Conclusion: The active substance induced local effects in the stomach of rats after repeated administration via gavage at ≥ 60 mg/kg bw (LOAEC 1.2%). The NOAEL is 20 mg/kg bw/day (NOAEC 0.4%).

4.7.3 Human data for RP 1:1 and RP 3:2

No human data are available for RP 1:1 and RP 3:2.

4.7.4 Comparison of RP 1:1 and RP 3:2

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.7-3 Comparison of the active substance and its components

Parameters	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Oral exposure effects Target organs Study duration Species LOAEL in mg/kg bw/day NOAEL in mg/kg bw/day	Gavage (corn oil) Local effects larynx, pharynx & oesophagus 90 days Rat 80 (LOAEC 3.2%) 30 (NOAEC 1.2%)	Gavage (corn oil) Mainly local effects Stomach 90 days Rat 60 (LOAEC 1.2%) 20 (NOAEC 0.4%)	Via drinking water local effects 2 years Rat 82 (m) or 109 (f) (0.19%) 15 (m) or 21 (f) (0.026%)
Dermal exposure Study duration Species LOAEL (mg/kg bw/day) NOAEL (mg/kg bw/day)	No data Local effects expected	No data Local effects expected	Local effects *, data not sufficient for assessment
Inhalation exposure effects target organs Study duration Species LOAEC (mg/m³) NOAEC (mg/m³)	No data Local effects expected	No data Local effects expected	Local effects - eye irritancy long term (lit. review) human 0.12

*: limited validity

4.7.5 Summary and Discussion of repeated dose toxicity

The NOAELs for the RP 1:1, RP 3:2 and formaldehyde reported in oral subchronic or chronic studies are in the same dose range (see table 4.7-3). For all compounds irritation at the site of contact is the main effect. However, related to the concentration in vehicle (corn oil) the RP 1:1 has a slightly higher NOAEC/LOAEC than the RP 3:2. Also in the acute toxicity studies effective concentration levels were slightly higher in the RP 1:1, though this is difficult to interpret since the dominant toxicological mechanism seems to be local corrosion (see Section 3.2.3).

Although the data on 2-hydroxypropylamine are of limited validity, there is some indication that the toxic effects of 2-hydroxypropylamine after repeated oral or inhalation exposure occurred at much higher dose levels. Therefore they do not impact the derivation of the overall NOAEL.

For the RP 3:2 also a developmental toxicity study is available indicating a LOAEL/NOAEL of 45/5 mg/kg bw d and a LOAEC/NOAEC of 2.25 / 0.25%. Considering the reduced exposure time and the different dose spacing of this developmental study compared to the 90 day study, the NOAEL/NOAEC of the 90 day study is considered as most relevant for risk assessment.

In summary for the risk assessment the LOAELs/NOAELs and LOAECs/ NOAECs from the 90 day studies will be taken into consideration: RP 1:1 – 80/30 mg kg bw d and 3.2/1.2%; RP 3:2 - 60/20 mg/kg bw d and 1.2 / 0.4%. These LOAELs7LOAECs refer to local effects in the upper gastro-intestinal tract. No systemic effects were detected.

No data are available on dermal exposure of the active substances. A dermal study is, however, not considered as reasonable due to the corrosive properties of the compound.

No data are available on inhalative exposure of the active substances. An inhalative study is, however, not considered as reasonable due to the corrosive properties of the compound. Inhalative exposure will expectedly be largely to the hydrolysis product formaldehyde, which is sufficiently investigated. The threshold of 0.12 mg/m³ for formaldehyde will be applied for assessing the risk from inhalation exposure.

4.7.6 Comparison with criteria for STOT RE

For RP 1:1 and RP 3:2 data on repeated dermal application are lacking. However, due to the corrosive properties of RP 1:1 and RP 3:2 a repeated dose toxicity study with dermal application is not justified. Chronic studies are available for formaldehyde these studies indicated local effects at the site of contact.

No repeated dose inhalation studies with RP 1:1 or RP 3:2 are available. However based on the hydrolysis study and the toxicokinetic study it is plausible that by dilution by the reaction of formaldehyde with biological media the equilibrium mixture quickly shifts towards formaldehyde. 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 RP 1:1 and RP 3:2 as well as the hydrolysis product formaldehyde induced local effects at the site of contact, i.e. in the gastro-intestinal tract. The LOAELs were 80 mg/kg bw day and 60 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.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

4.8.1.1 In vitro data – RP 1:1

Table 4.8-1 RP 1:1 Genotoxicity in vitro

Test system Method Guideline	**Organism/ strain(s)**	**Concentra-tions tested**	**identity as given in study report**	**Result**		**Remark**	**Reference**
				+ S9	- S9		
Salmonella microsome assay, OECD 471	S. typhimurium TA1535, TA1537 TA98, TA100, TA102	18.7, 37.5, 75, 150, 300 µg/plate	Grotan WS Batch 1025145 FA 26.4-28% HPA 68-71%	?	?	Negative test results with and without S9-mix but not tested up to cytotoxicity threshold. Invalid positive control with TA102 +S9-mix.	Schülke & Mayr (2000); DocIIIA6.6.1/01
Salmonella microsome assay, OECD 471	S. typhimurium TA1535, TA1537 TA98, TA100, E. coli WP2uvrA-	0.005, 0.015, 0.050, 0.150, 0.3, 0.5, 1.5, 5 mg/plate	OS 157338	+?	-	Reproducible positive results in TA100 with S9-mix, but the increase in revertants is less than 2-fold of the concurrent control	**Lubrizol Corporation (2000); DocIIIA6.6.1/02**
Chromosome aberration test; OECD 473	Chinese hamster lung (CHL) cells	1.8, 3.6, 7.3, 14.5, 22, 29, 58, 87, 116 µg/ml	OS 157338	+	+	Dose-dependent clastogenic activity and induction of polyploidy	**Lubrizol Corporation (2001); DocIIIA6.6.2**
Mammalian cell gene mutation test; OECD 476	Mouse lymphoma L5178Y TK+/- 3.7.2c cells	2.5, 5, 10, 20, 40, 60, 80 µg/ml	OS 157338	+	+	Dose-dependent mutagenic activity; predominantly clastogenic (small colonies)	**Lubrizol Corporation (2001); DocIIIA6.6.3/01**
Mammalian cell gene mutation test; OECD 476	Mouse lymphoma L5178Y TK+/- 3.7.2c cells	2.5, 5, 10, 20, 30, 40 µg/ml Grotan WS	Grotan WS Batch 1035116	+	+	Dose-dependent mutagenic activity; predominantly clastogenic (small colonies)	**Schülke & Mayr (2002); DocIIIA6.6.3/02**

?: ambiguous test results; +?: weak mutagenic activity

In the Salmonella microsome assay (OECD guideline 471) only weak mutagenic activity was detected (cf. DocIIIA6.6.1/02). A slight increase above historical and concurrent negative control values was found in TA100 with metabolic activation. A second Salmonella microsome assay has limited validity since the test substance was not tested up to cytotoxicity threshold (Schülke & Mayr, 2000, cf. DocIIIA6.6.1/01).

In the chromosome aberration test (OECD guideline 473; cf. DocIIIA6.6.2) dose dependent clastogenic as well as aneugenic activity was demonstrated both with and without metabolic activation.

In the mouse lymphoma assay detecting gene mutation as well as clastogenic properties the test substance gave positive results. More small colonies than large colonies were counted in this assay indicating predominantly clastogenic activity of the test substance (cf. DocIIIA6.6.3/01). These results were confirmed in a second independent mouse lymphoma assay (cf. DocIIIA6.6.3/02).

4.8.1.2 In vitro data – RP 3:2

Table 4.8-2: RP 3:2 Genotoxicity in vitro

Test system Method Guideline	**organism/ strain(s)**	**concentra-tions tested**	**identity as given in study report**	**Result**		**Remark**	**Reference**
				+ S9	- S9		
Salmonella microsome assay, OECD 471	S. typhimurium TA98, TA100, TA1535, TA1537	0, 1, 5, 10, 50, 100 µg/plate Mar71	Mar71 Batch PA 3622 Purity > 95%	-	-	Not tested up to cytotoxicity threshold; no 5th strain tested. Ambiguous test results	Schülke & Mayr (1997); DocIII A6.6.1/01
Salmonella microsome assay, OECD 471	S. typhimurium TA98, TA100, TA102, TA1535, TA1537	0, 12.5, 25, 50, 100, 200 µg/plate GrotaMar71	GrotaMAR71 Batch 1024828 FA 46.9% HPA 80.2%	-	-	Cytotoxicity threshold not reached. Ambiguous test results	Schülke & Mayr (2000); DocIII A6.6.1/02
Salmonella microsome assay, OECD 471	S. typhimurium TA98, TA100, TA1535, TA1537 & E. coli WP2uvrA-	0, 5, 15, 50, 150, 300, 500, 750, 1500 µg/plate	OS 157339	+	+	Positive results in TA98, TA100, and WP2uvrA also at non-cytotoxic concentrations. But only weak mutagenic activity	**Lubrizol Corporation (2000); DocIII A6.6.1/03**
Chromosome aberration test; OECD 473	Chinese hamster lung (CHL) cells	0, 2.5, 5, 7.5, 10, 20 µg/ml	OS 157339	+	+	Clastogenic activity also at non-cytotoxic dose levels.	**Lubrizol Corporation (2001); DocIII A6.6.2**
Mouse lymphoma assay; OECD 476	Mouse lymphoma L5178Y TK+/- 3.7.2c cells	0, 1, 2, 4, 8, 16, 32 µg/ml	GrotaMar 71 Batch 1042038	+	+	Mutagenic activity also at non-cytotoxic dose levels; predominantly clastogenic.	**Schülke and Mayr (2002); DocIII A6.6.3/01**
Mouse lymphoma assay; OECD 476	Mouse lymphoma L5178Y TK+/- 3.7.2c cells	0, 1, 2, 4, 8, 16, 24 µg/ml	OS 157339	+	+	Mutagenic activity also at non-cytotoxic dose levels; predominantly clastogenic.	**Lubrizol Corporation (2001); DocIII A6.6.3/02**

In the Salmonella microsome assay according to OECD 471 (Schülke & Mayr, 1997 & 2000, cf. DocIIIA6.6.1/01 & DocIIIA6.6.1/02) the test substance did not induce gene mutation in bacteria with and without metabolic activation. However, the test substance was not tested up to the cytotoxicity threshold limiting the validity of these studies. In a 3rd Salmonella microsome assay (Lubrizol Corporation, 2000, cf. DocIIIA6.6.1/03; OECD guideline 471) an increased number of revertants was detected in TA98, TA100, and WP2uvrA with and without metabolic activation also at non-cytotoxic concentrations. But this increase was maximal 2-fold of the concurrent control indicating only weak mutagenic activity.

In the chromosome aberration test (OECD guideline 473; Lubrizol Corporation, 2001, cf. Doc IIIA6.6.2) the test substance has clastogenic activity and induces polyploidy even at non-cytotoxic concentrations with and without metabolic activation. Accordingly, predominantly chromosome mutagenic activity (increase in small colonies) was demonstrated in two independent mouse lymphoma tests with and without metabolic activation (Schülke and Mayr, 2002, cf. DocIIIA6.6.3/01; Lubrizol Corporation, 2001, cf. DocIIIA6.6.3/02).

Conclusion: The active substance has weak mutagenic activity in the Salmonella microsome assay and chromosome mutagenic activity in mammalian cells.

4.8.1.3 Comparisons of in vitro data for RP 1:1, RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.8-3 Comparison of RP 1:1, RP 3:2 and its components

Parameters	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Gene mutation in bacteria	Weakly mutagenic	Weakly mutagenic	Mutagenic
Chromosome aberration in eukaryotic cells	Clastogenic	Clastogenic	Clastogenic ≥ 7.5 µg/ml
Gene mutation in mammalian cells	Mutagenic (mainly clastogenic)	Mutagenic (mainly clastogenic)	Mutagenic
DNA damage in bacteria and eukaryotic cells	No data	No data	Genotoxic
Overall assessment	Mutagenic activity in vitro	Mutagenic activity in vitro	Mutagenic activity in vitro

MA: metabolic activation

4.8.1.4 In vivo data – RP 1:1

Table 4.8-4 RP 1:1 Genotoxicity in vivo

Type of test Method/ Guideline	**Species Strain Sex no/group**	**Frequency of application**	**sampling times**	**dose levels**	**identity as given in study report**	**Results dose, sampling time and result +/-/±**	**Remarks**	**Reference**
Mouse bone marrow micronucleus test; OECD 474	Mouse NMRI 5 m & 5 f	Single i.p. application	24 h and 48 h after injection	10, 50, 100 mg/kg bw	Contram 121 Batch 24774	10 mg/kg bw, 24 h: - 50 mg/kg bw, 24 h: - 100 mg/kg bw, 24 h: - 100 mg/kg bw, 48 h: -	PCE/NCE ratio reduced in high dose (though PCE/NCE not statistically evaluated); minor clinical signs in high dose	**Becker Chemie (2002); DocIII A6.6.4/01**
Mammalian	Mouse	Single i.p.	24 h and 48	10, 50,	Contram	10 mg/kg	No historical	**Becker**

bone marrow chromosome aberration test; OECD 475	NMRI 5 m & 5 f	applica-tion	h after injection	100 mg/kg bw	121 Batch 24774	bw, 24 h: ± 50 mg/kg bw, 24 h: + 100 mg/kg bw, 24 h: + 100 mg/kg bw, 48 h: +	control; no statistical evaluation; documentation deficiencies; MTD questionable (no clinical symptoms; mitotic index not measured)	**Chemie (2002); DocIII A6.6.4/02**
Mammalian bone marrow chromosome aberration test; OECD 475	Mouse Swiss 5 m & 5 f	2 oral applica-tions (gavage, interval 24 h)	24 h after the last application	106, 212, 425 mg/kg bw	Grotan WS Batch 1025145 FA 26.4-28% HPA 68%-71%	106 mg/kg bw, 24 h: - 212 mg/kg bw, 24 h: - 425 mg/kg bw, 24 h: -	MTD not reached (mitotic index not reduced, no clinical symptoms)	**Schülke & Mayr (2000); DocIII A6.6.4/03**

±: ambiguous; MTD: maximal tolerable dose: PCE/NCE: polychromatic erythrocytes/normochromatic erythrocytes

Three studies are available which are able to detect systemic chromosome mutagenic activity in the bone marrow of mice.

In the mouse bone marrow micronucleus test according to OECD guideline 474 (2002, cf. DocIIIA6.6.4/01) no clastogenic or aneugenic activity was reported after i.p. injection of up to 100 mg/kg bw.

In a chromosome aberration study (cf. DocIIIA6.6.4/02) there are indications for clastogenic activity in the mouse bone marrow after i.p. injection of ≥ 50 mg/kg bw. However the study has deficiencies: No historical control, no statistical evaluation and documentation deficiencies. Another mouse bone marrow chromosome aberration test according to OECD guideline 475 (cf. DocIIIA6.6.4/03) was negative after oral application of up to 425 mg/kg bw. Neither in the i.p. study nor in the oral study the MTD was reached in terms of clinical symptoms. Furthermore the mitotic index was not analysed in the i.p. study and in the oral study it was not reduced.

In summary there is low concern for aneugenic or clastogenic effects in the bone marrow. Since there is limited confirmation that the active substance reached the bone marrow in terms of reduced PCE/NCE ratio or mitotic index the absence of genotoxic effects in bone marrow may also be due to the toxicokinetics of the formaldehyde releaser, expectedly formaldehyde release at first site of contact.

4.8.1.5 In vivo data – RP 3:2

Table 4.8-5 RP3:2 Genotoxicity in vivo

Type of test Method/ Guideline	**Species Strain Sex no/group**	**frequency of application**	**sampling times**	**dose levels in mg/ kg bw**	**identity as given in study report**	**Results give dose, sampling time and result +/-/±**	**Remarks**	**Reference**
Cytogenetic study; OECD 475	Mouse Swiss 5 m & 5 f	2 applica-tions via ga-vage, time interval 24h	24 h after the last applica-tion	0, 92, 183, 367	GrotaMAR 71 Batch 102828 FA 46.9% HPA 80.2%	ambiguous 92 mg/kg bw, 24 h: - 183 mg/kg bw, 24 h: - 367 mg/kg bw, 24 h: ±	no historical control; MTD not reached: (mitotic index not reduced, no clinical signs)	**Schülke & Mayr (2000); DocIIIA6.6.4/01**
Micronuc-leus test; OECD 474	Mouse NMRI 5 m & 5 f	Single application via gavage	24 or 48 h	0, 30, 100, 300	Contram MBO Batch 24773 FA 42.28%	negative 30 mg/kg bw, 24 h: - 100 mg/kg bw, 24 h: - 300 mg/kg bw, 24 h: - 300 mg/kg bw, 48 h:-	Clinical symptoms at high dose but PCE/NCE ratio not affected	**Bode Chemie (2002); DocIIIA6.6.4/02**

±: inconclusive

In the cytogenetic study presented by Schülke & Mayr (2000; cf. DocIIIA6.6.4/01; OECD guideline 475) a slight increase in %aberrant cells was observed at the highest dose but this effect was not statistically significant and no historical controls are presented. The authors concluded that the test result was negative. It might be questioned, whether the maximum tolerated dose was reached in this study since 1) all animals were found to be without clinical symptoms after exposure and 2) no decrease in mitotic index was observed. No details were given about the determination of the MTD. In conclusion, ambiguous test results were presented in this study.

In a micronucleus test according to OECD guideline 474 (Bode Chemie, 2002, cf. DocIIIA6.6.4/02) no increase in the number of micronuclei at a dose level up to 300 mg/kg bw, the maximum tolerated dose in terms of clinical symptoms. The PCE/NCE ratio was not affected.

In summary there is low concern for aneugenic or clastogenic effects in the bone marrow. Since there is no confirmation that the active substance reached the bone marrow in terms of reduced mitotic index or PCE/NCE ratio the absence of genotoxic effects in bone marrow may also be due to the toxicokinetics of the formaldehyde releaser, expectedly FA release at first site of contact.

4.8.1.6 Comparisons of in vivo data for RP 1:1, RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.8-6 Comparison of the RP 1:1, RP 3:2 and its components

Parameters	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Systemic genotoxicity	one test with deficiencies showing some indications for clastogenic effects ; two tests with negative outcome;. limited confirmation that a.s. reached bone marrow	One ambiguous result (cytogenicity bone marrow); one negative result (micronucleus test), limited confirmation that a.s. reached bone marrow	Negative (cytogenetic & micronucleus assay) contradictory results in humans
Local genotoxicity	No data (but see positive in vitro data)	No data (but see positive in vitro 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)

4.8.2 Human information

No human data are available for the RP 1:1 or the RP 3:2. Human data for the hydrolysis product formaldehyde see table 4.9-1 above and specific documents.

4.8.3 Summary and discussion of mutagenicity

Studies on the RP 1:1 and RP 3:2 gave evidence for mutagenic activity in vitro, predominantly clastogenic effects were detected. It is considered that the genotoxicity is related to the hydrolysis product formaldehyde which is assumed to be hydrolysed in the aqueous medium of in-vitro tests. The DNA-protein cross-linking activity of formaldehyde is a possible mechanism. No indication for mutagenicity of 2-hydroxypropylamine has been detected in available bacterial studies and no structural alerts are present (confirmed by OECD toolbox: Benigni/Bossa rulebase, DNA-binding; Cramar rules and CAESAR mutagenicity model).

The RP 1:1 and RP 3:2 were applied at doses above 100 mg/kg bw, but the MTD was not reached in all experiments. Though there are some ambiguous positive results the total database supports that the active substance is not easily systemically available and is not genotoxic distant from the site of first contact. Data on the hydrolysis product formaldehyde suggested more local than systemic mutagenic effects. Formaldehyde is genotoxic in vitro and it induces local clastogenic effects in vivo. Similar results could be expected for the active substance in high concentrations in aqueous environment.

Consequently -for both of the formaldehyde releasers considered here- low concern for germ cell mutagenicity is assumed.

4.8.4 Comparison with criteria

Based on the available data and mechanistic considerations of formaldehyde release local genotoxic effects are to be expected from RP 1:1 and RP 3:2. The presently available data for RP 1:1, RP 3:2, 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, there is no need for this further information. Therefore, labeling for mutagenicity according EU Regulation 1272/2008/EC is not 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 RP 1:1 and RP 3:2 on the data of the hydrolysis product formaldehyde. Arguments for and against reading across the carcinogenicity data and C&L conclusion from formaldehyde to RP 1:1 and RP 3:2 are listed in chapter 4.9.4. 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.

4.8.5 Conclusions on classification and labelling

Classification for mutagenicity category 2 is required.

4.9 Carcinogenicity

4.9.1 Non-human information for the RP 1:1 and the RP 3:2

No long-term carcinogenity studies on experimental animals are available for any of the 2 substances.

4.9.2 Human information

No human data are available for the RP 1:1 or the RP 3:2. Human data for the hydrolysis product formaldehyde see table 4.9-1 above and specific documents.

4.9.3 Comparison of the RP 1:1, the RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.9-1 Comparison of the active substance and its components

Parameters	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Systemic carcinogenicity in experimental animals	No data	No data	No carcinogenic activity
Local carcinogenicity in experimental animals	No data	No data	Carcinogenic activity after inhalation at > 7.4 mg/m³
Systemic carcinogenicity in humans	No data	No data	Conflicting results
Local carcinogenicity in humans	No data	No data	Conclusion from not unequivocal epidemiological studies: increased tumour risk after inhalation exposure

4.9.4 Summary and discussion of carcinogenicity

In summary it is considered that the equilibrium of 2-hydroxypropylamine and formaldehyde (1:1 or 3:2 reaction products) 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. The available repeated dose studies with the reaction products of 2-hydroxypropylamine and formaldehyde (1:1 or 3:2) indicate predominantly local effects. Furthermore the tests for systemic genotoxicity were negative for both of the 2-hydroxypropylamine: formaldehyde reaction products (1:1 and 3:2). The hydrolysis products formaldehyde and HPA are unlikely to induce systemic genotoxicity as demonstrated by respective negative genotoxicity tests and (for HPA) QSARs. Also the carcinogenicity studies for formaldehyde are negative.

Consequently it is to be expected that the reaction products of 2-hydroxypropylamine and formaldehyde (1:1 and 3:2) show 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) Proposal by the eMS: 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) Proposal by the applicant for the European Biocidal Products Regulation: 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 the 1:1 and 3:2 ratio based on "total releasable formaldehyde" or "free formaldehyde" content

supportive arguments for proposal 1: Classification according to releasable Formaldehyde, i.e. Skin Corr. 1, Skin Sens 1, Carc. 1B	**supportive arguments for proposal 2:** Classification according to "free Formaldehyde", i.e. Skin Corr. 1
Risk through formaldehyde-release in water is covered	Classification usually relates to the substance itself and not to potential release or degradation products which occur during different use scenarios
According to CLP Regulation Annex I, paragraph 1.1.1.3 a WoE evaluation is required for 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 < 1 hour. Each mg RP 1:1 releases 0.28 mg formaldehyde, each RP 3:2 releases 0.45 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 **à** 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

4.9.5 Comparison with criteria

Genotoxiciy data for the RP 1:1 and RP 3:2 support local genotoxicity, but no systemic genotoxicity. No carcinogenicity studies are available for the RP 1:1 or the RP 3:2. 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.. Arguments supporting classification in Category 1B and arguments for non-classification are listed above. Based on a total WoE evaluation it is proposed to base classification of the RP 1:1 and the RP 3:2 on the data of the hydrolysis product formaldehyde.

4.9.6 Conclusions on classification and labelling

Classification for carcinogenicity, category 1B is proposed.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information – RP 1:1

Two 90-day studies on repeated dose toxicity according to OECD 408 in rats have been performed (see 3.5 and A6.4.1). In these subchronic gavage studies pathological examinations included also reproductive organs in males and females. No treatment related effects were observed in these organs at dose levels of 150 mg/kg bw (Doc IIIA 6.4.1/01) and 250 mg/kg bw (Doc IIIA 6.4.1/02). However, in the latter study (Schülke & Mayr, 2002, cf. DocIIIA6.4.1/02) the MTD was not reached.

4.10.1.2 Non-human information – RP 3:2

Table 4.10-1 Summary of data for potential fertility effects

Route of exposure	**Testtype Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**identity as given in study report**	**LOAEL Parental; F1**	**NOAEL Parental; F1**	**Reference**
gavage	OECD 415	Rat/ Wistar HanRcc 24males and 24females/group	Pre-Pairing: 70 days Pairing: 14 days maximum Gestation: ~ 21 days Lactation: 21 days	0, 5, 15, and 45 mg/kg bw/day in corn oil corresponding to 0, 0.1%, 0.3%, 0.9% (w/w)	Grotan OX Batch 1129974 Purity 90-100%	Parental local = 15 mg/kg bw corr. to 0.3%: histopath. in forestomach Parental systemic = 45 mg/kg bw: ↓ male food consumption and bw gain F1: 45 mg/kg bw: ↑ sum of post-implantation and post-natal loss	Parental local = 5 mg/kg bw corr. to 0.1%: Parental systemic = 15 mg/kg bw F1: 15 mg/kg bw	**Lubrizol Deutschland GmbH & Schülke & Mayr GmbH 2009, Doc IIIA6.8.2**

A valid subchronic study on repeated oral dose toxicity according to OECD 408 in rats has been performed (Bode Chemie, 2002, cf. DocIIIA6.4.1/02; see also Section 3.5). In this gavage study pathological examinations included also reproductive organs in males and females. No treatment related effects were observed in these organs even at a dose level of 120/180 mg/kg bw/day, a dose inducing severe local effects in the stomach and systemic effects secondary to the ulcerative gastritis & peritonitis.

A fertility study according to OECD TG 415 was carried out (Lubrizol Deutschland GmbH & Schülke & Mayr GmbH 2009, Doc IIIA6.8.2) and indicated histopathological changes in the forestomach of males in the mid dose group of 15 mg/kg bw (0.3% a.s.) leading to a local oral NOAEL of 5 mg/kg bw with 0.1% a.s. (weight/weight). With 45 mg/kg bw in addition to local stomach effects also reduced male food consumption and bw gain were observed as well as an increased sum of post-implantation and post-natal loss. Consequently a systemic NOAEL of 15 mg/kg bw for parents as well as F1 was derived from this study.

As discussed in detail in Doc III-A 6.8.2.2 the latter finding should not be considered as direct substance related effect. The lack of concomitant findings in the fertility study and the developmental study is considered the strongest support for this conclusion: No increase of post partum toxicity in terms of clinical signs, body weight or other histopathological findings was observed in the fertility study and also in the developmental study no increase in post-implementation loss, or resporptions or malformations, were observed up to the MTD of 90 mg/kg bw (see Doc III-A 8.1). Consequently no classification for developmental toxicity is proposed.

4.10.1.3 Human information

No human data are available.

4.10.1.4 Comparison of the RP 1:1, the RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in specific documents.

Table 4.10-2 Comparison of RP 1:1, RP 3:2 and its components

Type of study	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Repeated dose toxicity (≥ 90 days)	Rat, oral No effects on reproductive organs (mainly local effects)	Rat, oral No effects on reproductive organs (mainly local effects)	Different species, oral or inhalation: dominant local effects.
Special studies on fertility	No data	Rat, oral, One-generation reproduction toxicity study (OECD guideline 415): dominant parental local effects with local NOAEL of 5 mg/kg bw ~ 0.1% and systemic parental and F1 NOAEL of 15 mg/kg bw	No data

4.10.2 Developmental toxicity

4.10.2.1 Non-human information – RP 1:1

No data are available on the developmental toxicity of the reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1). However, the reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2) hydrolyses to the 1:1 reaction product and developmental toxicity of the 3:2 reaction product is sufficiently investigated. Developmental toxicity of the 3:2 reaction product occurred in rabbits after gavage application only at dose levels inducing severe maternal toxicity.

4.10.2.2 Non human information RP 3:2

Table 4.10-3 Developmental toxicity study of RP 3:2

Route of exposure	**Testtype Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses per day**	**identity as given in study report**	**Critical effects dams fetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL Teratogenicity Embryotoxicity**	**Reference**
Oral Gavage	OECD guideline 414	Rabbit Himala-yan female 24	Gestation day 6-28	0, 5, 45, 90, 135 mg/kg bw	GrotaMar 71 Batch 1094394 Purity 99%	Local effects in the stom-ach No terato-genicity	5 mg/kg bw/day	90 mg/kg bw/day	**Lubrizol Deutschland GmbH (2006); DocIIIA6.8.1**

In a study on teratogenicity in rabbits according to OECD guideline 414 (see Table 3.8.1; Lubrizol Deutschland GmbH, 2006, cf. DocIIIA6.8.1) rabbits were gavaged with 0, 5, 45, 90, 135 mg/kg bw/day corresponding to a concentration of 0, 0.25, 2.25, 4.5, 6.75% in corn oil. A dose of 135 mg/kg bw/day resulted in severe maternal toxicity like a decrease in body weight, increased mortality and abortions. Necropsy revealed local lesions in the stomach of dams and an increased incidence in dilatation of the renal pelvis. The authors of the study suggested a NOAEL for maternal toxicity at 90 mg/kg bw/day. However, there is some evidence that at least an increased incidence of lesions in the stomach occurred also at 45 mg/kg bw. Developmental toxicity like an increased number of early and late resorptions, a decreased number of foetuses, an increase in post-implantation loss and mortality of foetuses was only observed at 135 mg/kg bw/day, a dose which resulted also in severe maternal toxicity. No increase in the incidence of retardations, variations or malformations was detected in any treatment group.

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.

4.10.2.3 Human information

No human data are available.

4.10.2.4 Comparison of the RP 1:1, the RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.10-4 Comparison of the RP 1:1, RP 3:2 and its components

Exposure route	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Dermal exposure	No data	No data	No data but corrosive properties
Inhalation	No data	No data	Maternal effects in rats LOAEL 39 ppm (47 mg/m³) NOAEL 20 ppm (24 mg/m³) developmental effects LOAEL 39 ppm (47 mg/m³) NOAEL 20 ppm (24 mg/m³)
Oral exposure	No data	Maternal effects in rabbits LOAEL 45 mg/kg bw/day NOAEL 5 mg/kg bw/day developmental effects NOAEL 90 mg/kg bw/day LOAEL 135 mg/kg bw/day	Maternal effects in mice LOAEL 185 mg/kg bw/day NOAEL 148 mg/kg bw developmental effects LOAEL 185 mg/kg bw NOAEL 148 mg/kg bw/day

4.10.3 Summary and discussion of reproductive toxicity

The reaction product from paraformaldehyde and 2-hydroxy¬propylamine (RP 3:2) have no effects on reproductive organs in subchronic repeated dose toxicity studies; a one-generation reproduction toxicity study with the RP 3:2 according to OECD guideline 415 showed dominant local effects and no effects sufficient for classification for reproductive toxicity. A study on fertility with the RP 1:1 is not expected to provide additional toxicological information since the RP 3:2 hydrolyses to the RP 1:1 and finally to HPA and formaldehyde.

Data on formaldehyde suggested that this hydrolysis product may affect – if at all – reproductive organs only as a consequence of dominant local effects. In contrast, the data base on the hydrolysis product 2-hydroxypropylamine is sparse and systemic bioavailability is not excluded. However, in comparison to the other components the data on repeated dose toxicity of 2-hydroxypropylamine (although of limited validity) suggested that toxic effects of 2 hydroxypropylamine occurred at much higher dose levels.

No data are available on developmental toxicity of the RP 1:1. The RP 3:2 induced developmental effects only at dose levels resulting in severe maternal toxicity, presumably mainly from local effects on the gastro-intestinal tract after oral exposure. Similarly, formaldehyde has developmental effects but only at dose levels with severe local maternal toxicity after inhalation or oral exposure. No data are available on 2-hydroxypropylamine. However, in comparison to the other components the data on repeated dose toxicity of 2-hydroxypropylamine (although of limited validity) suggested that toxic effects of 2-hydroxypropylamine occurred at much higher dose levels.

In summary, there is no evidence for adverse effects of the RP 3:2 on embryo and foetal development at dose levels inducing no local maternal toxicity. Since in biological systems the RP 3:2 hydrolyses to the RP 1:1 and finally to HPA and formaldehyde and there is no evidence for adverse developmental effects for HPA or for Formaldehyde it is concluded that also for the RP 1:1 there is no concern for developmental toxicity.

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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.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity- RP 1:1

The subchronic rat study according to OECD guideline 408 summarized in **HPT-DocIII A6.4.1** included also functional observations. This functional observation battery included changes in autonomic activity, gait, posture, response to handling, as well the presence of abnormal movements or behaviour. Sensory reactivity to different types of stimuli (auditory, visual, proprioreceptive) was measured and assessment of grip strength performed. In the last week of the study additionally the motor activity was tested in an "Auto track" animal activity meter. Furthermore, detailed clinical observations were made once a week. No effects of neurotoxicological relevance were reported. Also the other subchronic rat study (Schülke & Mayr, 2002, **cf. DocIIIA6.4.1**/02) included functional observations and did not show respective specific effects. However the study is not considered as valid.

4.11.1.2 Neurotoxicity – RP 3:2

In a subchronic rat study according to OECD guideline 408 summarized in **MBO-DocIIIA6.4.1/02** the test substance induced mainly local effects in the stomach at a dose level of ≥ 60 mg/kg bw. The functional observation battery included autonomic activity, gait, posture, response to handling, the presence of

abnormal secretions, abnormal movements or behaviour. At the end of the exposure period (>= week 11) functional observations were recorded including sensory reactivity to different types of stimuli (auditory, visual, proprioreceptive), assessment of grip strength and motor activity. Only in the high dose group that was beyond the MTD (mortality 3/10 males, 5/10 females) adverse effects as piloerection (all animals), ataxia (one female) and reduced pupil size (3/7 m and 5/5 f survivors) was detected.

4.11.1.3 Comparison of RP 1:1, RP 3:2 and its components

Detailed data on formaldehyde and 2-hydroxypropylamine are presented in the specific documents.

Table 4.11-1 Comparison of RP 1:1, RP 3:2 and its components

	Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Effects	90 day, gavage rat No neurotoxic effects detected	90 day, gavage, rat Reduced pupil size LOAEL 180/120 mg/kg bw/day (above MTD) NOAEL 60 mg/kg bw/day	Rat, inhalation exploratory behaviour and learning affected with LOAEL = 0.12 mg/m³, but considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health is unlikely

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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 relevant neurotoxicological effects are evident at doses below the MTD.

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 to key studies are highlighted bold throughout this chapter.

Please note that formaldehyde data have 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 2-hydroxypropylamine further information is attached in the Appendix “2-Hydroxypropylamine” with Robust Study Summaries for key studies.

5.1 Degradation

5.1.1 Stability

Hydrolysis

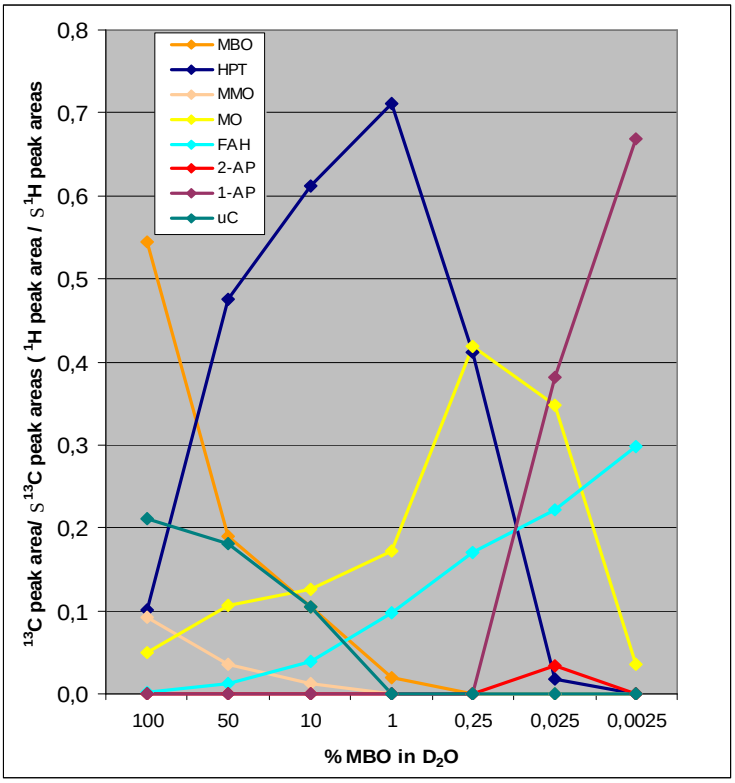
Table 5.1.1-1 Hydrolysis of the active substance

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
Non-guideline study	**4, 7, 9**	**20°C**	**1% (w/w)**	**not applicable**	**< 1 h**	**not applicable**	**MBO - Doc III A7.1.1.1.1**

Hydrolysis in water - Summary and Conclusion (**MBO - Doc III A7.1.1.1.1)**

The hydrolysis of CONTRAM™ MBO was studied using ^{1}H and ^{13}C-NMR technique (see Doc. II-A 7.1.1.1.1, Study A 7.1.1.1.1). Thereby, the dependence of pH, concentration and composition of hydrolysis products has been investigated. Spectra were measured from unbuffered D_2O solutions at 25°C in equilibrium revealing different CONTRAM™ MBO concentrations ranging from 0.0025% (v/v) to 100%. The composition of the solutions in D_2O was found to be strongly dependent on the concentration. While at 100% the main constituent is MBO, its content decreased with higher dilutions and was absent at 0.25%. The content of α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) increased up to a dilution of about 1%, but decreased at lower concentrations. Formaldehyde and 5-methyloxazolidine were identified as products of hydrolysis, the content of both compounds increased when dilution increased. At the highest dilution (0.0025% (v/v)), the active substance was almost completely hydrolysed to formaldehyde hydrate and 2-hydroxypropylamine (see Figure 5.1.1-1 and Table 5.1.1.-2).

Fig 5.1.1-1: Concentration and composition of hydrolysis products



MBO: N,N’-methylene-bis-(5-methyloxazolidine)
HPT: hexahydro-1,3,5-tris(2-hydroxypropyl)-s-triazine
MMO: N-methylol-5-methyloxazolidine
MO: 5-methyloxazolidine
FAH: formaldehyde hydrate
2-AP: 2-aminoproanol(1)
1-AP: 1-aminopropanol(2)
uC: unknown compounds

Table 5.1.1-2 Composition of hydrolysis products

Compound	Contram ™ MBO in D_2O_2						
	100% (v/v)	50% (v/v)	10% (v/v)	1% (v/v)	0.25% (v/v)	0.025% (v/v)	0.0025% (v/v)
	Signal area/Σ signal areas						
MBO	0.5437	0.1903	0.1045	0.0199	0	0	0
Trianzine (HPT)	0.1012	0.4751	0.6124	0.7108	0.4109	0.0170	0
MMO	0.0930	0.0350	0.0133	0	0	0	0
MO: 5-methyloxazolidine	0.0501	0.1060	0.1260	0.1723	0.4181	0.3472	0.035
2-AP: 2-aminoproanol(1)	0	0	0	0	0	0.0340	0
1-AP: 1-aminopropanol(2)	0	0	0	0	0	0.3808	0.6680
FAH: formaldehyde hydrate	0.0018	0.0123	0.0388	0.0969	0.1710	0.2210	0.2974
uC: unknown compounds	0.2102	0.1813	0.1050	0	0	0	0

In a further test (**MBO - Doc III A7.1.1.1.1)** the time-dependent formation of Formaldehyde was measured in buffered aqueous solutions containing 1% w/w at different pH values (4, 7 and 9) at 25°C. The highest degree of formaldehyde formation was observed under acidic conditions at pH 4 corresponding also to the highest degree of degradation of Grotan® WS. The lowest amount of formaldehyde was measured at pH 9. It

was found that at pH 4 and 7 the formaldehyde content reached a plateau after ca. 1-2 hours; while at pH 9 the reaction was slower, reaching the plateau after 3-4 hours.

Table 5.1.1-3: Dependence of pH

pH 4		pH 7		pH 9	
time [h]	% H_2CO	time [h]	% H_2CO	time [h]	% H_2CO
0.33	38.27	0.33	25.83	0.33	7.18
0.92	39.48	0.92	27.00	0.92	7.48
2.10	41.35	2.10	27.15	2.10	8.39
3.67	41.71	3.67	27.65	3.67	9.20
5.23	41.76	5.23	28.67	5.23	9.10
6.80	41.66	6.80	28.82	7.58	9.05

Conclusion:

The study demonstrates that the equilibrium of hydrolysis is strongly dependent on the concentration in water. The test results reveal that at concentration levels being expected in the environment, CONTRAM™ MBO is assumed to be completely hydrolysed to Formaldehyde and 1-aminopropanol (2-Hydroxypropylamine). As the equilibrium was reached within a few hours in the performed test investigating a 1% w/w solution, the hydrolysis half-life DT_{50} is expected to be less than 1 hour at all pH values under environmentally relevant conditions (temperature, concentration, and pH). The study is summarized in the following Table 5.1.1-3.

Table 5.1.1-4 Hydrolysis of Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)

Guideline / Test method	**pH**	**Temperature [°C]**	**Initial TS concentration [% v/v]**	**Results**	**Reference**
Non-guideline study, no GLP	**---**	**25°C**	**0.0025, 0.025, 0.25 1, 10, 50, 100**	**High degree of hydrolysis at env. relev. concentrations**	**Doc. III-A 7.1.1.1.1 Study A 7.1.1.1.1**
	4, 7, 9	**20°C**	**1 % w/w**	**Fast kinetic: equilibrium within 1-2 h at pH 4 and 7 and 3-4 h at pH 9**	
	Conclusion DT_{50}< 1 h under environmentally relevant conditions				

Photolysis in water

There is no study on photolysis of Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2) in aqueous solution available as explained in Doc. III-A 7.1.1.1.2 (Justification for non-submission). The UV spectrum indicates no absorption of light at wave-lengths > 290 nm (see Doc III-A 3.4). The US EPA method OPPTS 835.2210 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 cannot undergo direct photolysis in sunlight. Therefore, the active substance is no candidate for noteworthy photolysis in sunlight and the performance of a test is not necessary. The available information is assumed to be sufficient.

Phototransformation in air

Table 5.1.1-5 Phototransformation in air for the main constituent MBO

Guideline / Test method	Molecule / radical	Rate constant	Molecule/Radical concentration	Half-life ($\tau_{1/2}$)	Reference
Estimation direct photolysis	**h υ**	**0 (expected)**	**-**	**-**	**Doc III-A 7.1.1.1.2 Justification for non-submission**
Estimation indirect photolysis (Calculation AopWin v1.91)	**OH**	**3.13 · 10-10 cm3/molecule s**	**0.5 · 10^6 / cm^3 (24 h-day)**	**1.23 h**	**Doc III-A 7.3.1**
	Ozone	**Negligible compared to reaction with OH radicals**	**-**	**-**	
	NO_3	**Negligible compared to reaction with OH radicals**	**-**	**-**	

The reaction rate of 3,3’-methylene-bis [5-methyl-oxazolidine], the main constituent of CONTRAM™ MBO, with OH-radicals in the atmosphere was calculated using AopWin v1.91 (see Doc. III-A 7.3.1). The calculated half-life was 1.23 hours corresponding to an OH-radical concentration of $5x10^5$ radicals per cm^3 (recommended default value according to EC 2003, part II, chapter 3, 2.3.6.3, p.51). In the gas phase, MBO is rapidly degraded in air via reaction with OH radicals, degradation by nitrate and ozone is considered to be comparatively negligible. The UV spectrum shows no absorption of light at wave-lengths > 290 nm (**see** 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. Due to the low volatility of the main constituent N,N’-methylene-bis(5-methyloxazolidine), this degradation pathway is expected to be of minor importance.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available

5.1.2.2 Screening tests

Ready biodegradability tests

RP 3:2

The available biodegradation studies using the active substance “reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)” as test substance are presented in Table 5.1.2-1.

Table 5.1.2-1 Biodegradation of the active substance

Guideline / Test method	Test type	Para-meter	Inoculum		Additional substrate	Test substance concentr.	Degradation		Reference
			Type	Concen-tration			Incubation period	Degree [%]	
OECD 301D GLP Klimisch 2	**ready**	**BOD; ThOD**	**Sewage effluent, soil micro-org.**	**0.4 ml/L**	**no**	**1.37 mg/L Contram™ MBO***	**28 d**	**56%**	**MBO – Doc III A 7.1.1.2.1/01**
OECD 301D GLP Klimisch 2	**ready**	**BOD; COD**	**River water**	**0.2 ml/L**	**no**	**2 mg GrotaMar 71®/L**	**28 d**	**30.2%**	**MBO – Doc III A 7.1.1.2.1/02**

* according to a statement by the applicant.

The biodegradability of the active substance (presumable Contram™ MBO) was investigated in 3 studies on ready biodegradability according to OECD Guideline 301D (Closed-Bottle-Test) or OECD Guideline 306 (Biodegradability in seawater – Closed-Bottle-Method).

In the first study (**MBO – Doc III A 7.1.1.2.1/01**) a mixture of sewage effluent and soil microorganisms was used as inoculum. The $BOD/ThOD_{NO3}$ ratio was found to be 56% after 28 days. When nitrification would not be considered, the $BOD/ThOD_{NH3}$ is calculated to 77%. In both cases the pass-level was not reached within 14 days. The test item was not toxic to the microorganisms due to the positive result of the toxicity control.

In the second Closed-Bottle-Test (**MBO – Doc III A 7.1.1.2.1/02)** using GrotaMar® and river water as inoculum a BOD/COD ratio of 30.2% was calculated. The measured BOD was corrected by the theoretical oxygen consumption due to formation of nitrate and nitrite which were measured simultaneously. Since the reference substance potassium hydrogen phthalate reached 84% degradation after 28 days and meets the 10-d-window the inoculum is considered as suitable, though the biological and nutritional status of the river water was not characterised.

The interpretation of the biodegradation tests performed with the active substance is complicated by the fact that actually a mixture of substances is tested. According to the OECD Guidelines, tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals. Studies on hydrolysis (**cf. MBO - Doc III A7.1.1.1**) indicate that in aqueous media the hydrolysis products are present. Both formaldehyde and 2-hydroxypropylamine, the end-products of hydrolysis, are readily biodegradable. The used concentrations in the ready tests were in the range of approximately 0.02 to 0.04%. During the present studies probably intermediates were formed which were degraded at slower rates than the end-products. However no information is available to quantify if the negative ready biodegradation results is attributed to more stable intermediates or the limitations of the OECD ready biodegradability test protocol for complex mixtures. A justification for non-submission of a test on inherent biodegradability (MBO - Doc III A7.1.1.2.2) was accepted based on the above mentioned arguments.

Formaldehyde

Table 5.1.2-2 Biodegradation 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**	**microorganis ms from a digester of a STP with predominantly 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, cf. Table 5.1.2 2). 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) . 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 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). No test on inherent biodegradability is required.

Based on the available information, formaldehyde is classified as "readily biodegradable, fulfilling the 10-d window". Thus, the following rate constants for biodegradation shall be applied in the environmental exposure assessment according to EU Technical Guidance Document (TGD) on Risk Assessment (EC, 2003), Part II, Chapter 2.3.6.4, Table 6 and Chapter 2.3.6.5, Tables 7 and 8, respectively:

- $k = 1\ h^{-1}$ for biodegradation in sewage treatment plants;
- $k = 4.7 \times 10^{-2}\ days^{-1}$ for surface water;
- $k = 2.3 \times 10^{-2}\ days^{-1}$ for soils.

2-Hydroxypropylamine

No study on ready biodegradability was submitted by the applicant (cf. HPA – Doc IIIA7.1.1.2.1 justification). Therefore to predict the biodegradation BioWin v4.1 of the EPI SUITE TM[4] was run. The results indicate readily biodegradability.

A further QSAR calculation was perform with VEGA v1.0.8[5] with the protonated form of 2-hydroxypropylamine (cf. Doc III A 7.1.1.2.1 QSAR VEGA). Also this model predicts reliable results that ready biodegradability is possible. Limitations concerns that only moderately similar compounds with known experimental values in the training set have been found and the accuracy of prediction for similar molecules found in the training set is not optimal.

However the findings of the predictions are in line with OECD (2011)[6] that concluded that 2-hydroxypropylamine is readily biodegradable. Also the Chemical Safety Report on 1-aminopropan-2-ol (=2-hydroxypropylamine) provides experimental evidence that this chemical is readily biodegradable. 2-Hydroxypropylamine was not harmonised classified as dangerous for the environment according to Annex VI of 67/548/EWG though the acute effect value for algae is between 10 – 100 mg/L.

In addition, there are other studies/ reviews available dealing with biodegradation. Degradation of 2-hydroxypropylamine in a BOD test resulted in 4% degradation after 5 days when a non-acclimated inoculum was used (Bridie et al., 1979a). In two further BOD tests degradation were in the range of 38-46% after 20 days (Davis and Carpenter, 1997). With adapted inoculum (inherent biodegradability) 43% degradation were obtained within 5 days (Bridie et al., 1979a). Davis and Carpenter (1997) listed the results from a Zahn-Wellens Test that indicate 44% DOC removal after 24 days.

In anaerobic serum bottle degradation studies, 2-hydroxypropylamine exhibited a lag period of 9 days followed by a removal rate of 22 mg/L/day; during the observation period, 65% of initial test substance was removed (HSDB, 2014) [7].

[4] http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[5] http://vega.marionegri.it/wordpress/resources/qsar-in-silico-tools/

[6] OECD (2011): SIDS INITIAL ASSESSMENT PROFILE, C1 -13 Primary Amines, http://webnet.oecd.org/Hpv/UI/handler.axd?id=300f88e7-dd00-4c98-95be-d8f5077bb9e4 2013-12-12

[7] http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:%2278-96-6%22, 2014-01-08

Biodegradability in seawater

Table 5.1.2-3 Biodegradation of the reaction product RP 3:2 in seawater

Guideline / Test method	Para-meter	Test medium	Addit. substrate	Test substance concentr.	Degradation		Reference
					Duration	Degree	
OECD 306- Closed Bottle No GLP, Klimisch 2	**BOD/ COD**	**Costal seawater**	**yes**	**2.5 / 5.0 mg/L GrotaMar 71®**	**28 d**	**69.4 / 63% at day 22, 53.8% at day 28**	**MBO – Doc III A 7.1.1.2.3**

In a test on biodegradation in seawater according to OECD 306 (Closed-Bottle-Method) BOD/COD ratios of 69 and 54% were obtained at test substance concentrations of 2.5 and 5 mg/L (cf. Table 5.1.2-3). The results refer to biological oxidation without considering nitrification. The pass level was reached within 14 d at both concentrations, therefore there is a potential for biodegradation in the marine environment.

Two validity criteria of the test were not met: Oxygen consumption indicating a high load of DOC though prior aging of the seawater exceeded 30%; t_{50} of sodium sodium benzoate was greater than 4 days indicating poor microbial activity of the seawater. Also for the higher test concentration the difference of extremes of replicate values of TS removal at plateau (at the end of test) was above 20%.

Despite the deficiencies the test demonstrates that the active substance (reaction product) has a potential for ultimate biodegradation in the marine environment.

5.1.2.3 Simulation tests

No data available.

5.1.3 Summary and discussion of degradation

Two closed bottle tests on ready biodegradability (OECD guideline 301D) of the reaction product of paraformaldehyde and 2-hydroxy-propylamine (ratio 3:2) (Contram™ MBO and GrotaMar 71®) confirmed that the test item was not readily biodegradable though biodegradation reached between 30% and 77% depending on the consideration of nitrification.

The interpretation of the biodegradation tests performed with the active substance is complicated by the fact that actually a mixture of substances is tested. According to the OECD Guidelines, tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals. Studies on hydrolysis (cf. section above) indicate that in aqueous media the hydrolysis products are present. Formaldehyde, one end-products of hydrolysis, is readily biodegradable on the basis of results from a study according to OECD 301A. Concerning the second product 2-hydroxypropylamine no study on ready biodegradability has been submitted, but evidence on its ready biodegradability has been provided.

The dominant degradation process for 2-hydroxypropylamine in the environment is expected to be biodegradation. There a several lines of evidence available that indicate that 2-hydroxypropylamine is readily biodegradable: Predicted results from two QSAR models indicate that the substance is readily biodegradable. Evidence presented in an international assessment (OECD, 2011) as well as other scientific findings confirm that 2-hydroxyprobylamine is susceptible to biodegradation, though study design and results vary. Overall, 2-hydroxypropylamine appears to be readily biodegradable.

The used concentrations in the ready tests with Contram™ MBO and GrotaMar 71® were in the range of approximately 0.02 to 0.04%. During the present studies probably intermediates were formed which were degraded at slower rates than the end-products. However no information is available to quantify if the negative ready biodegradation results is attributed to more stable intermediates or the limitations of the OECD ready biodegradability test protocol for complex mixtures.

GrotaMar 71® reached a positive result in a biodegradation test with seawater in a non GLP OECD guideline study 306, closed bottle procedure. Therefore the reaction product of paraformaldehyde and 2-hydroxy-propylamine (ratio 3:2) has the potential for ultimate biodegradation in the marine environment.

Hydrolysis is the dominant removal mechanism for the reaction product paraformaldehyde and 2-hydroxypropylamine (ratio 3:2). The equilibrium of hydrolysis is strongly dependent on the concentration in water. The test results reveal that at concentration levels being expected in the environment, CONTRAM™ MBO is assumed to be completely hydrolysed to formaldehyde and 2-hydroxypropylamine. As the equilibrium was reached within a few hours in the performed test investigating a 1% w/w solution, the hydrolysis half-life DT50 is expected to be less than 1 hour at all pH values under environmentally relevant conditions (temperature, concentration, and pH).

The reaction product is considered to be rapid degradable because it is demonstrated that primary degradation via hydrolysis in the aquatic environment has a half-life <16 days (corresponding to a degradation of >70 % within 28 days). It is shown that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment as shown by their harmonized classification (cf. Part A, Table 1.2-2) that lacks a classification for aquatic hazards.

Photolysis is not relevant for abiotic degradation of the reaction product.

In the gas phase, the reaction product is rapidly degraded in air via reaction with OH radicals with a calculated half-life of 1.23 hours, degradation by nitrate and ozone is considered to be comparatively negligible.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Adsorption of the UVCB substance (CONTRAM™ MBO) was estimated according to the Draft OECD TG 121 using HPLC in GLP-conform, Klimisch 2 rated study (**cf. MBO - Doc III A7.1.3**).

Because of hydrolysis, analytical determination of the distribution coefficient for the main constituent N,N'-methylene-bis(5-methyloxazolidine) was not possible. Thus the more stable hydrolysis product 5-methyl-oxazolidine was chosen as analytical target compound.

As a result the retention time of the target compound 5-methyl-oxazolidine was always identical with the dead times of the analytical system measured using Thiourea. Therefore, for the Koc only an upper limit could be estimated. The Koc value was estimated to be ≤1 L/kg according to the study authors.

The study failed to determine the Koc value of the test substance. However the Koc of 5-methyl-oxazolidine should be lower than the value for Thiourea. The measured Koc value for Thiourea according Schuurman et al. (2006)[8] is 7.1 and an estimation of 2.75 was made by Environment & Health Canada, 2008[9].

Because no experimental Koc could be determined a QSAR estimate was calculated using the Soil Adsorption Coefficient Program (KOCWIN v2.0, EPI Suite v4.11) that estimates the soil adsorption

[8] Schuurmann, G., R. Ebert and R. Kuhne. 2006. Prediction of the sorption of organic compounds into soil organic matter from molecular structure. Environ. Sci. Technol. 40:7005-7011

[9] http://www.ec.gc.ca/ese-ees/CE2D78C6-9635-494E-9513-17D5D0C0223D/batch2_62-56-6_en.pdf

coefficient (Koc) of organic compounds. Corrected Koc values for 5-methyl-oxazolidine were 6.5 L/kg (MCI Method) and 5.6 L/kg (log Kow method), both indicating very low adsorption.

The QSAR calculation for nonhydrophobics according to the TGD (2003) for the reaction product results in a Log Koc of 0.99 (=9.77 L/kg).

Because 5-methyl-oxazolidine has a similar molecular structure as N,N’-methylene-bis(5-methyl-oxazolidine), the main component of the active substance (cf. Chapter 1), the estimated Koc value determined for 5-methyl-oxazolidine (6.5 L/kg) can be adopted.

Conclusion:

The low adsorption coefficient (< 7 L/kg) indicates that the reaction product is highly mobile in soils and will not adsorb onto sewage sludge and sediment solids to any significant extent.

5.2.2 Volatilisation

Table 5.2.2-1: Vapour pressure

Vapour pressure	EC method A.4 calculated	GrotaMar 71; Batch-no.: 1024828 Formaldehyde: 46.9% 2 hydroxypropylamine: 80.2%	5.83 hPa (25°C), calculated from regression curve	**Doc. III-A 3; Study A 3.2/01**
	EC method A.4 calculated	3,3'-methylenebis[5-methyloxazolidine]; Lot No.: 24773 Content of easy releasable formaldehyde: 43.52% w/w Content of total formaldehyde: 42.28% w/w	2 Pa (20°C); 2.8 Pa (25°C); 13.9 Pa (50°C); calculated The calculated vapour pressure vales are extrapolated.	**Doc. III-A 3; Study A 3.2/02**
	calculated with epi suite3.12	n.a.	0.014 hPa (25°C)	**Doc. III-A 3; Study A 3.2/03**
Henry´s Law Constant	Epi Suite 3.12 HENRYWIN v3.10	n.a.	0.011 Pa x m^3/mol (calculated with EpiSuite 3.12)	**Doc. III-A 3; Study A 3.2.1**

The transfer of a substance from the aqueous phase to the gas phase is estimated by means of its Henry´s Law constant. The calculated Henry´s law constant for N,N’-methylene-bis(5-methyloxazolidine) (0.011 Pa m^3 $mole^{-1}$, **cf. MBO – DOC III A3.2.1**) indicates that this main constituent is not volatile from aqueous solutions.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

RP 3:2

There are no experimental data about bioaccumulation available. Because of the hydrolysis properties of the reaction product (**cf. MBO - Doc III A7.1.1.1.1**) experimental determination of the BCF is not possible (MBO – Doc III A7.4.2 – Justification).

According to the 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 Kow value for N,N'-methylene-bis(5-methyloxazolidine), the main constituent of the UVCB substance, was determined to be in the range of -0.043 to 1.89. Thus the value is outside of the domain of the QSAR.

According to ECHA (2012)[10] the effect of hydrolysis may be a significant factor for substances discharged mainly to the aquatic environment: the concentration of a substance in water is reduced by hydrolysis so the extent of bioconcentration in aquatic organisms would also be reduced. Where the half-life, at environmentally relevant pH values (4-9) and temperature, is less than 12 hours, it can be assumed that the rate of hydrolysis is greater than that for uptake by the exposed organisms. The DT50 for the reaction product of para-formaldehyde and 2-hydroxy-propylamine (ratio 3:2) was determined to be less than one hour. Therefore the likelihood of bioaccumulation is greatly reduced and the determination of a BCF value is not necessary in this specific case.

The QSAR model for the estimation of a terrestrial bioconcentration factor is applicable to a logKow range of 1 to 6. The BCF - logKow relationship applies generally to neutral organic substances which are not easily biotransformed (EC, 2003, part III, p. 41). Therefore no valid QSAR calculation for terrestrial bioconcentration can be made for the reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2) for GrotaMar® 71 (log Kow -0.043 to -0.6). The log Kow value for Contram™ MBO was determined to be 1.89. Because only experimentally derived BCF values are considered relevant for classification a calculated BCF value was not taken into account for classification. (cf. MBO – Doc III A7.5.5 – Justification for non-submission).

Formaldehyde

Because of a log Kow = 0.35 no valid QSAR estimation according to EC, 2003 can be made for aquatic and terrestrial bioconcentration. An experimental study with fish (cf. Doc III-A 7.4.3.3.1_HCHO) is not required.

As additional information in Doc III-A7.4.2./01_HCHO a test with two marine fish species (olive flounder, black rockfish) for formaldehyde residues is summarised. The fish were exposed to formaldehyde (0, 37, 111, and 185 mg L-1) for one hour (static system). Then they were transferred into clean water. After 0, 24, 48, and 72 hours, fish were sampled and formaldehyde residues in muscle tissues were determined (substance-specific analysis). Only in one case (highest concentration, immediately after exposure (0 h)), formaldehyde concentrations were significantly increased compared to control. Although it is not possible to derive a BCF_{fish} from the study, the results can be taken as an indication for the low potential for bioaccumulation of formaldehyde in marine organisms. Further studies on formaldehyde residues in several aquatic animals after short-term exposure (1 - 24 hours) are summarized in Doc III-A7.4.2./01_HCHO. An accumulation of formaldehyde was not observed in any species tested (cf. Appendix "Formaldehyde Core Dossier").

2-Hydroxypropylamine

The low octanol/water partition coefficient (log K_{ow} = -0.96) indicates a low bioaccumulation potential (cf. Appendix "2-Hydroxypropylamine"). The bioaccumulation of neutral compounds depends mainly on lipophilicity, whereas dissociation, the pH-dependent ion trap, and electrical attraction of cations impact the BCF of ions (Franco, 2010).

To establish the bioconcentration in aquatic organisms of 2-hydroxypropylamine, a search was conducted by the applicant in the following databases (HSDB 2007, ECOTOX 2007, MITI 2006) and a review (Davis & Carpenter 1997).

In the following an overview of these existing data is presented:

[10] ECHA (2012): Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance, http://echa.europa.eu/documents/10162/13632 /information_requirements_r7c_en.pdf , 2013-10-24

Based upon an experimental log K_{ow} of -0.96, the BCF for 2-hydroxypropylamine can be estimated to be 0.11 from a regression-derived equation. This BCF value suggests that the compound will not bioconcentrate significantly in aquatic organisms (Lyman 1982, cited in HSDB 2007).

The log BCF is reported to be -0.76 (Davis & Carpenter 1997). This would correspond to a BCF of 0.17.

According to the 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 2-hydroxypropylamine is outside of the domain of the QSAR. Therefore a QSAR estimate with the BCFBAF TM Epi Suite v4.11 model was performed. Please note that this model is applicable to ionic substances. The log BCF was calculated with 0.50 (= BCF of 3.16 L/k). The model (training set of non-ionic plus ionic substances) has a r^2 of 0.833 and a SD of 0.5.

Data on terrestrial bioconcentration were not described in the literature.

The QSAR model for the estimation of a terrestrial bioconcentration factor is applicable to a logKow range of 1 to 6. The BCF - logKow relationship applies generally to neutral organic substances which are not easily biotransformed (EC, 2003, part III, p. 41). Therefore no valid QSAR calculation for terrestrial bioconcentration according to the TGD methodology can be made for this at environmental relevant pH values charged metabolite. However, the log K_{OW} indicates a low potential for bioaccumulation.

OECD (2011) reported a measured BCF of 2.7-3.6 L/kg_{ww} for 2-propanol, 1-amino (OECD TG 305C) indicating that this molecule is not expected to be bioaccumulative.

5.3.1.2 Measured bioaccumulation data

No study on bioconcentration in aquatic organisms is performed.

5.3.2 Summary and discussion of aquatic bioaccumulation

In view of the rapid hydrolysis, a test on aquatic or terrestrial bioconcentration of the reaction product seems scientifically not justified. Also the use of a QSAR estimation for aquatic bioconcentration based on a log Kow of -0.043 to 1.89 that is outside the applicability domain is not scientifically sound. A bioaccumulation potential for the main constituent N,N’-methylene-bis(5-methyloxazolidine) could not be identified based on a very low log Kow value and a DT50 hydrolysis of <1 hour.

In experimental studies on bioaccumulation no elevated formaldehyde levels were found. Additional information on log Kow (0.35) support the experimental findings that formaldehyde does not accumulate in aquatic biota.

The metabolite 2-hydroxypropylamine is not expected to be bioaccumulative based on a low Kow value of

-0.96 and a predicted BCF of 3.16 L/kg_{ww}. Moreover an experimental determined BCF value from literature in the range of 2.7-3.6 L/kg_{ww} supports this finding.

5.4 Aquatic toxicity

The constituents of the reaction product (active substance) hydrolyse completely in concentrations which are expected to occur in waste waters and surface waters. Also in the media of toxicity tests the presence of hydrolysis products is expected (cf. Chapter 5.1.1). Therefore the observed effects are expected to be caused by a mixture of hydrolysis products.

Tables 5.4-1: Summary of relevant information on aquatic toxicity

See chapters 5.4.1, 5.4.2, 5.4.3, 5.4.4.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of the active substance (reaction product) to aquatic organisms was tested in several studies covering different trophic levels.

Table 5.4.1-1 Acute toxicity to fish

Guideline / Test method	Species/ Test material	Endpoint / Type of test	Exposure design	Exposure duration	Results [mg/L] [1] LC_0	LC_{50}	LC_{100}	Remarks	Reference
OECD 203 GLP, Klimisch 1	***Danio rerio*** **GrotaMar 71®**	**Mortality**	**Semi-static**	**96 h**	**44.4**	**57.7 Cl (51.5-64.6**	**100**	**NOEC 29.63 mg/L**	**MBO - Doc III A7.4.1.1/01**
OECD 203 GLP, Klimisch 1	***Danio rerio*** Contram™ MBO*	**Mortality**	**Semi-static**	**96 h**	**50**	**71 (geometric mean)**	**100**	**--**	**MBO - Doc III A7.4.1.1/02**
OECD 203 No GLP, Klimisch 2	*Scopthalmus maximus* GrotaMar 71®	Mortality	Semi-static	96 h	100	135	180	No monitoring of test subst.	MBO - Doc III A7.4.1.1/03

[1] results based on nominal concentrations

* according to a statement by the applicant.

The reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2, GrotaMar 71® and presumable Contram™ MBO) was tested twice with the zebra fish *Brachydanio rerio* in 96 h semi-static tests according to OECD Guideline 203 (cf. Table 5.4.1-1). In both studies the concentration of the active substance during exposure was monitored indirectly via formaldehyde, resulting in no significant loss of test substance during the test period (conc. ≥ 80% of nominal (via form¬aldehyde). Observation in the first study (**MBO - Doc III A7.4.1.1/01)** include immobilisation of fishes at the top or bottom of the tank at concentrations where mortality occurred. In addition at 44 mg/L one fish showed strong movements of gills.

The reaction product was additionally tested for acute toxicity to the juvenile turbot *Scophthalmus maximus*, a marine species, in a non GLP 96 h semi-static test according to the OECD Guideline 203 (cf. MBO - Doc III A7.4.1.1/03). This study resulted in a LC_{50} value of 135 mg/L (nominal concentrations). However no analytical measurements were performed. Deviations from the guideline include that the test organisms received constant dim illumination (no daily photoperiods) and the pH value was outside the recommended range for marine tests (>7.5 and <8.5) according to OPPTS 850.1075.

5.4.1.2 Long-term toxicity to fish

No data available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 5.4.2-1 Acute toxicity to invertebrates

Guideline / Test method	**Species/ Test item**	**Endpoint / Type of test**	**Exposure**		Results [mg/L][1]			**Reference**
			design	**duration**	**EC_0**	**EC_{50}**	**EC_{100}**	
OECD 202/I GLP, Klimisch 1	***Daphnia magna*** Contram™ MBO*	**Mobility**	**static**	**48 h**		**28**	**40**	**MBO - Doc III A7.4.1.2/01**
OECD 202/I GLP, Klimisch 1	***Daphnia magna Straus*** GrotaMar 71®	**Mobility**	**semi-static**	**48 h**	**17.2**	**37.9 (Cl 35 – 42)**	**55.6**	**MBO - Doc III A7.4.1.2/02**
ISO/TC147 /SC5/WG2/ N61 (draft) No GLP, Klimisch 3	*Acartia tonsa* GrotaMar 71®	Mobility	static	48 h		4.1		MBO - Doc III A7.4.1.2/03

[1] results based on nominal concentrations

* according to a statement by the applicant.

Two GLP tests on acute toxicity to *Daphnia magna* according to OECD Guideline 202 were conducted (cf. Table 5.4.2-1). The static test resulted in a 48 h-EC_{50} value of 28 mg/l (geometric mean between two concentrations causing 0% and 100% mortality), while in the semi-static system a 48 h-EC_{50} value of 37.9 mg/l was obtained. In both tests monitoring of the test substance concentration was performed during exposure (indirectly via formaldehyde) and the 80% limit, prescribed by the Guideline, was kept except for the lowest tested concentration of study MBO – Doc III A7.4.1.2/02.

Furthermore, the acute toxicity of the reaction product to the marine copepod *Acartia tonsa* was investigated according to the ISO proposal ISO/TC147/SC5/WG2/N61. After 48 h exposure an EC_{50} of 4.1 mg/l was established. However, control mortality was 20% after 48 h, and in the test no clear dose-response relationship could be established with the 24 h effect data. Also the test item was not analytically measured and physical characterisation of test water at the beginning and the end was not reported. Therefore, this test is considered to be valid with restrictions and used as supportive information.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 5.4.2.2-1 Chronic toxicity to invertebrates

Guideline / Test method	Species/ Test item	Endpoint / Type of test	Exposure		Results [mg/L][1]		Remarks	Reference
			design	duration	NOEC	LOEC		
OECD 211 GLP, Klimisch 1	***Daphnia magna*** Grotan Ox	**Reproduction**	**semi static**	**21 d**	**1.3**	**3.2**	**formaldehyde > 80% of nominal**	**MBO - Doc III A7.4.3.4**

[1]….: nominal concentration

A test on reproduction of *Daphnia magna* was performed with Grotan OX according to the OECD Guideline 211 in a semi static system (cf. Table 4.1.2-4). Test parameters were mortality, reproduction, the age at first reproduction and the size of the parent animals at the end of the test. The NOEC based on mean offspring of survivors was found to be 1.3 mg/L (EC_{10} 1.1 mg/L Cl 0-3 mg/L; EC50 26.4 mg/L, Cl 11.6-1608 mg/L; cumulative offspring of survivors). Test item related effects were found for the additional endpoints mobility (NOEC = 8.0 mg/L), intrinsic rate of population growth (NOEC > 50 mg/L), and age at first reproduction (NOEC > 20 mg/L). Length and diameter of the parent animals were not affected at 20 mg/L (determined after termination of exposure). However at 20 mg/L mortality of parent animals was 15% and 100% after 4 days at 50 mg/L.

Analytical measurements revealed that the formaldehyde content remained stable at >80% of the initial values over the exposure period. Therefore, the nominal values can be used for deriving the effect values.

5.4.3 Algae and aquatic plants

Table 5.4.3-1 Inhibition on algae

Guideline / Test method	Species/Test item	Endpoint / Type of test	Exposure		Results [mg/L]			Reference
			design	duration	NOE_rC	E_bC_{50}[1]	E_rC_{50}[2]	
OECD 201 GLP Klimisch 3	*Desmodemus subspicatus* Contram™ MBO*	Growth rate	static	72 h	0.9 (n.c.) 0.5 [3]	3.2 (n.c.) 1.8 [3]	4.3 (n.c.) 2.4 [3]	MBO - Doc III A7.4.1.3/01
OECD 201 GLP Klimisch 3	*Desmodemus subspicatus* GrotaMar 71®	Growth rate	static	72 h	2.2 (n.c.)	2.6 (n.c.)	5.7 (n.c.)	MBO - Doc III A7.4.1.3/02
ISO/TC147 /SC5/WG5 No GLP, Klimisch 3	*Skeletonema costatum (marine)* GrotaMar 71®	Growth rate	static	72 h	No data	No data	3.77 (n.c.)[3]	MBO - Doc III A7.4.1.3/03

* according to a statement by the applicant
[1] calculated from the area under the growth curve; [2] calculated from growth rate;
[3] nominal concentrations corrected for an average of 55% recovery
n.c.: nominal concentration
appr. … approximately

The reaction product was also tested twice for inhibition of algal growth with the species *Desmodesmus subspicatus.* Based on growth rate, NOECs of 0.5 and 2.2 mg/L and E_rC_{50} of 2.4 and 5.7 mg/L were obtained (cf. Table 5.4.3-1).

In study MBO - Doc IV A7.4.1.3/1 (cf. MBO - Doc III A7.4.1.3/1, see Figure 5.4.3-1 for detailed results) average recoveries from test solutions of the different concentrations was on average 55% (measured formaldehyde concentrations cf. Table 5.4.3-2). The same analytical method as in **MBO - Doc III A7.4.1.2/01** and **MBO - Doc III A7.4.1.1/02** was used. Because no raw data were reported, endpoints were estimated reflecting the measured concentrations (cf. Table 5.4.3-1) by correcting the calculated (nominal) E_rC50 by a 55% recovery. Because no raw data or results from individual incubation flasks have been reported, endpoints can only be estimated reflecting the measured concentrations.

Table 5.4.3-2 Actual concentrations of the test substance (analyte formaldehyde)

Nominal Concentration [mg/L]	**Measured Concentration [mg/L]**	
	$t_{(0)}$	**$t_{(72h)}$**
10	5.81	5.02
8	4.37	3.79
4	1.91	2.57
control	No peak detected	

Another weakness of the study is that the pH value was not maintained (in all concentration levels with algae including the control except the two highest levels (8 mg/L and 10 mg/L). No explanation is given in the study report that addresses the pH deviation of more than one unit. Also the control showed an increase from pH 8.53 to 10.83 (cf. Table 5.4.3-3).

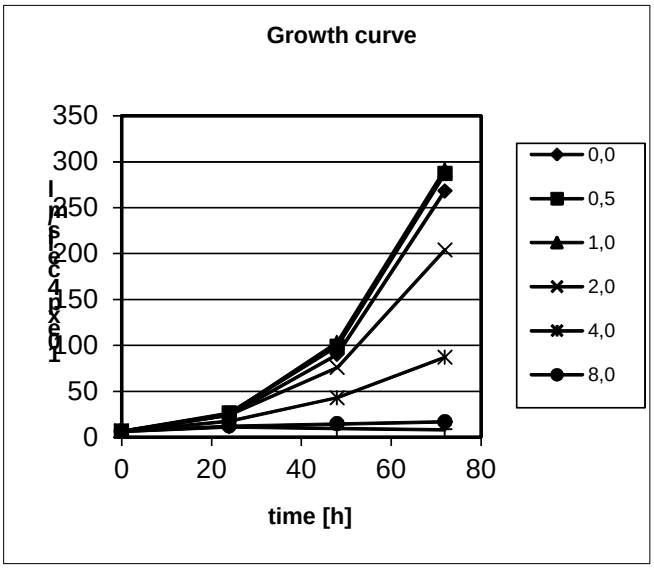
Table 5.4.3-3 Results of pH determinations

Test Solution	pH at t_0	pH at t_{72h}
Control with algal inoculation	8.53	10.83
Control without algal inoculation	8.50	8.22
10 mg/L with algal inoculation	8.46	8.15
0 mg/L without algal inoculation	8.44	8.09
8 mg/L with algal inoculation	8.51	8.12
8 mg/L without algal inoculation	8.39	8.07
4 mg/L with algal inoculation	8.36	9.46
4 mg/L without algal inoculation	8.19	7.98
2 mg/L with algal inoculation	8.24	10.47
2 mg/L without algal inoculation	8.13	8.24
1 mg/L with algal inoculation	8.19	10.40
1 mg/L without algal inoculation	8.02	8.33
0.5 mg/L with algal inoculation	8.16	10.59
0.5 mg/L without algal inoculation	8.03	8.35

According to OECD (2000)[11] growth of algal test cultures can cause increase of pH due to consumption of HCO_3 ions. Maintenance of stable pH when testing an ionised substance is therefore important to ensure that the balance between dissociated and non-dissociated forms of the substance is maintained. This balance was not maintained for the hydrolysis product 2-hydroxypropylamine with a pKa of 9.94. As was shown by Abeliovich and Azov (1976)[12] increased pH (≥8 facilitates penetration into green algae cells (*Scenedesmus obliquus*) of Methylamine (pKa=10.6, SRC PhysProp Database)[13], a related compound to 2-hydroxypropylamine causing disruption of photosynthesis. So pH related effects cannot be excluded for the tested mixture i.e. 2-hydroxypropylamine.

ECHA (2012)[14] recommended that if validity criteria of the test were not fulfilled (e.g. pH increase) only data from the part of the test where exponential growth occurs and the validity criteria for the controls are fulfilled should be used. However this was not possible for the current study since pH was only measured at t_0 and t_{72}. The validity criteria “cell concentration in control cultures increased at least by a factor of 16 within 3 days” was met.

Figure 5.4.3-1 Results of the algae test MBO - Doc III A7.4.1.3/01 (growth curve and cell concentration data)



Test substance Concentration (nominal/) [mg/l]	Cell concentrations (mean values) [10^4 cells/ml] measured 0 h	24 h	48 h	72 h	Inhibition Percent biomass	growth
control	6.37	24.2	90.2	268	0.0	0.0
0.5	6.55	26.4	99.1	287	-8.0	-1.1
1	6.51	25.4	103	291	-10.7	-1.6
2	6.51	24.1	75.9	204	19.9	7.8
4	6.44	17.5	43.0	87.0	62.1	30.4
8	6.44	12.0*	14.5	16.7	91.9	74.5
10	6.46	11.3	9.48	8.20	96.2	93.6
Temp. [°C]	22.5-23.0°C					
pH	8.02–8.53			7.98–10.83		

In study MBO - Doc IV A7.4.1.3/02 (MBO - Doc III A7.4.1.3/02) no information about the stability of the test item can be drawn from the study data. Analytical measurements of the formaldehyde content in the test item were not successful. During the test period an increase of aldehyde groups was observed which was probably caused by injection of aldehydes via the aeration system. Moreover it is unclear if the aldehyde contamination also occurred in the algae test vessels via the aeration system. The report only says that the aldehyde groups seem to be injected by the aeration system of the stability controls.

Additionally the inhibition of algal growth on the marine species *Skeletonema costatum* was investigated according to ISO/TC147/SC5/WG5 (adopted EN ISO 10253:2006) resulting in an E_rC_{50} of 3.77 mg/L (Cl

[11] OECD (2002) OECD SERIES ON TESTING AND ASSESSMENT Number 23: GUIDANCE DOCUMENT ON AQUATIC TOXICITY TESTING OF DIFFICULT SUBSTANCES AND MIXTURES. http://www.oecd-ilibrary.org/docserver/download/9750231e.pdf?expires=1385738495&id=id&accname=guest&checksum=90E189B53DA5CB93A8280F813D892394, 2013-11-8

[12] Abeliovich A, Azov Y. 1976: Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol. Jun;31(6):801–806

[13] http://esc.syrres.com/fatepointer/webprop.asp?CAS=74895, 2013-12-12

[14] ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.7b: Endpoint specific guidance. http://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf , 2013-11-8

3.43-4.16) after 72 h. However, reduction of the growth rate was dependent on time. It was greatest after 24 hours (EC_{50} = 0.52 mg/L) followed by some recovery of the test organisms. In the control vessels logarithmic growth was observed. Also the pH of study MBO - Doc IV A7.4.1.3/03 (MBO - Doc III A7.4.1.3/03) differed more than one unit as allowed by the guideline in the control. The variation coefficient for the controls was not reported and no analytical measurements were performed. Thus two out of three validity criteria of the adopted ISO guideline have not been fulfilled.

Based on a weight of evidence approach enough experimental information is presented to evaluate the toxicity to freshwater and marine algae. All three studies indicate comparable results, thus the outcome of the individual studies is strengthen though each study has some major deficiencies (cf. Klimisch scores in Table 5.4.3-1). Another line of evidence is that algae have been shown to be the most sensitive species in aquatic acute toxicity tests. The chronic Daphnia study according to OECD Guideline 211 resulted in a NOEC based on mean offspring of survivors of 1.3 mg/L. Therefore a NOEC algae below 1.3 mg/L is plausible. It can be anticipated that a new study will not reveal different findings. The studies on the toxicity towards algae demonstrate that the reaction product of para-formaldehyde and 2-hydroxy-propylamine (ratio 3:2) was acutely toxic to the test organisms. Moreover the study results indicate that the reaction product is harmful to aquatic life with long lasting effects.

5.4.4 Other aquatic organisms (including sediment)

Inhibition of microbial activity (aquatic)

Table 5.4.4-1 Inhibition of microbial activity (aquatic)

Guideline / Test method	Test item	Species / Inoculum	Endpoint / Type of test	Exposure		Results [mg/L]			Reference
				design	duration	NOEC	EC_{50}	EC_{80}	
OECD 209 No GLP, Klimisch 2	Contram™ MBO*	Activated sludge, municipal	Inhibition of respiration	static	3 h	16 (n.c.)	44 (n.c.)	82 (n.c.)	MBO - Doc III A7.4.1.4/1
OECD 209 No GLP, Klimisch 1	**Mar 71**	**Activated sludge, municipal**	**Inhibition of respiration**	**static**	**3 h**		**44**		**MBO - Doc III A7.4.1.4/2**
OECD 209 GLP, Klimisch 2	**nominal conc. Grota Mar 71®**	**Activated sludge, industrial**	**Inhibition of respiration**	**static**	**3 h**		**10.43**		**MBO - Doc III A7.4.1.4/3**

* according to a statement by the applicant, n.c . nominal concentration

The acute toxicity of the reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2) towards bacteria was tested according to OECD Guideline 209 in 2 studies by determining the inhibition of respiration in sludge samples from biological treatment plants receiving predominantly domestic sewage (cf. Table 5.4.4-1). In both tests, the 3 h-EC_{50} was established at a concentration of 44 mg/L. In one study the NOEC was determined to be 16 mg/L.

In a third study using sludge from an industrial treatment plant a 3 h-EC_{50} of 10.43 mg/L was obtained. According to the TGD (2003) if the substance under consideration is relevant for industrial and municipal STPs the toxicity assessment should be conducted for both kinds of STPs separately. A $PNEC_{microorganisms}$ should be obtained as a first step in the effects assessment for microorganisms in both domestic and industrial sewage treatment plants.

Taken into consideration the results of the two OECD 209 non GLP studies that resulted in an EC50 of 44 mg/L it is clearly showed that no adaption of the inoculum occurred. The GLP study is much better reported

and the method of the EC50 derivation is based on Probit analyses instead of graphical examination compared to the other two studies.

The results are comparable to the other reported values given the variability of the observed results from the method i.e. EC50 is in the range of 10 to 100 mg/L.

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

CLP:

Aquatic Acute 1:

Aquatic acute toxicity: L(E)C_{50} values for all three trophic levels >1 mg/L;

Lowest L(E)C_{50} value: E_rC_{50} (algae) =2.4 mg/L

- **è No classification**

Studies used:

- Doc. III-A 7.4.1.1/01: Arbeitsgemeinschaft GAB Biotechnologie GmbH und IFU Umweltanalytik GmbH (1995) Acute Toxicity Testing of MAR 71 in Zebra-fish (*Brachydanio rerio*) under Semi-static Conditions. -> **LC_{50} (fish) =58 mg/L**
- Doc. III-A 7.4.1.2/01: Institut Fresenius (2000), OECD 202, Part I Study on the Acute Toxicity towards Daphnia of "3,3'- Methylene-bis [5-methyl-oxazolidine]" -> **EC_{50} (crustacea) =28 mg/L**
- Doc. III-A 7.4.1.3/01: Institut Fresenius (2000), OECD 201, Study on the toxicity towards algae of "3,3'- Methylene-bis [5-methyl-oxazolidine]" -> **E_rC_{50} (algae) =2.4 mg/L**

Aquatic Chronic Categories:

The reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2) is rapidly degradable, adequate chronic toxicity data are available for cladocerans and algae. The algae NOE_rC is 0.5 mg/L, which leads to a classification with Aquatic Chronic 3.

The result from the algae study was used for classification based on a weight of evidence. The major deficiency of the study (rated Klimisch 3) was the high variability of the pH value in the control and in some test concentrations (no individual data of the test vessels were reported in the study). Therefore toxicity might be confounded by this variability. However two studies with the same species showed comparable results (E_rC50 of 2.4 and 5.7 mg/L).). In the second green algae study (A 7.4.1.2/02) guideline conform pH variations were reported. Also a third study on marine algae confirmed the findings from the freshwater algae tests (E_rC50 of 3.8 mg/L).

Another line of evidence is that algae have been shown to be the most sensitive species in aquatic acute toxicity tests. The chronic Daphnia study according to OECD Guideline 211 resulted in a NOEC based on mean offspring of survivors of 1.3 mg/L. Therefore a $NOEC_{algae}$ below 1.3 mg/L is plausible.

For fish only short term toxicity values in the range of 10 – 100 mg/L are available, which in combination with a log K_{ow} <1.89 would not lead to a classification.

Aquatic Chronic 1:

- **è No classification**

Aquatic Chronic 2:

- **No classification**

Aquatic Chronic 3:

- **Classification with Aquatic Chronic 3**

Studies used:

- Doc. III-A 7.1.1.1.1**:** Preiss A. (2008), comparable to OECD 111, Hydrolysis of the equilibrium mixture of hexahydro-1,3,5-tris(2.hydroxypropyl)-s-triazine and N,N-methylene-bis-(5-methyloxazolidine) -> **DT50< 1 h under environmentally relevant conditions**
- Doc. III-A 3: Partition coefficient of the reaction product, OECD 117 -> **log K_{ow} = -0.043 – 1.89**
- Doc. III-A 7.4.1.1/01: Arbeitsgemeinschaft GAB Biotechnologie GmbH und IFU Umweltanalytik GmbH (1995) Acute Toxicity Testing of MAR 71 in Zebra-fish (*Brachydanio rerio*) under Semi-static Conditions. -> **LC_{50} (fish) =58 mg/L**
- Doc. III-A 7.4.1.2/01: Institut Fresenius (2000), OECD 202, Part I Study on the Acute Toxicity towards Daphnia of "3,3'- Methylene-bis [5-methyl-oxazolidine]" -> **EC_{50} (crustacea) =28 mg/L**
- Doc. III-A 7.4.1.3/01: Institut Fresenius (2000), OECD 201, Study on the toxicity towards algae of "3,3'- Methylene-bis [5-methyl-oxazolidine]" -> **NOE_rC_{50} (algae) =0.5 mg/L,**

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

6 CLP:

Proposed classification and labelling according to Reg. (EU) No 1272/2008, Annex VI, Table 3.1 and Reg. (EU) No 286/2011

Classification and Labelling			**Justification**
GHS Pictograms		-	No classification for acute toxicity is proposed since for all three tropic levels $L(E)C_{50}$ values > 1mg/L are available. Chronic Toxicity: Rapidly degradable substance for which adequate chronic toxicity data are available for daphnia and algae. Lowest chronic value is the NOE_rCs from algae with 0.5 mg/L -> Aquatic Chronic 3.
Signal words		-	
Classification		Aquatic Chronic 3	
Hazard statements		H412: Harmful to aquatic life with long lasting effects	
Precautionary Statements	**General**	-	
	Prevention	P273: Avoid release to the environment	
	Response	-	
	Storage	-	
	Disposal	P501: Dispose of contents/container in accordance with local/regional/national/ international regulations (to be specified).	

7 OTHER INFORMATION

Not available

8 REFERENCES

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
A2.6/01	2007	Contram MBO - Method of manufacture of the active substance, Lubrizol Hamburg Lubrizol Deutschland GmbH, M. Gierschmann, M. P. Scholz, 15.11.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.6/02	2007	Manufacture of GrotaMar 71 Schülke & Mayr GmbH, S. Hendrich, 7.11.2007 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.7/01	2007	Purchased material specifications sheet, Product: Contram MBO/BC6120. Lubrizol Deutschland GmbH, 16.11.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.7/02	2007	Release specification of GrotaMar 71. Schülke & Mayr GmbH, S. Hendrich, 13.11.2007 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.7/03	2007	Determination of the Formaldehyde content of different batches CONTRAM™ MBO: Oxazolidine, 3,3`-methylenebis[5-methyloxazolidine], (CAS# 66204-44-2) Quality Control Laboratory – Lubrizol Deutschland GmbH, Document No. 57, 17.12.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.7/04	2007	Formaldehyde content of different batches of GrotaMar 71 Schülke & Mayr GmbH, S. Hendrich, 13.11.2006 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.7/05	2004	Spektroskopische Untersuchungen zum Produktvergleich Contram MBO/ GrotaMar 71 Fraunhofer ITEM (Dr A Preiss), Draft Report, 9/04 non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A2.7/06	2005	13C NMR-Untersuchungen zum Produktvergleich II GrotaMar 71/Contram MBO Fraunhofer ITEM (Dr A Preiss), Report 29.8.05 non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A2.7/07	2008	Hydrolysis of the equilibrium mixture of hexahydro-1,3,5-tris(2.hydroxypropyl)-s-triazine and N,N-methylene-bis-(5-methyloxazolidine)	Y	Schülke & Mayr + Lubrizol

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		Fraunhofer ITEM (Dr A Preiss), Report 25.2.08 non GLP, unpublished		
A2.10_01	2007a	Medical statement for formaldehyde-releasing active ingredients GPL not applicable, unpublished	Y	Lubrizol
A2.10_01	2007b	Statement of compliance to all maximum permissible workplace exposures GPL not applicable, unpublished	Y	Lubrizol
A2.10_01	2007	Medical statement for Formaldehyde-releasing active ingredients GPL not applicable, unpublished	Y	Schülke & Mayr
A2.10/02	2007	Estimation of the Environmental Concentrations and the Preliminary Environmental Risk Assessment of “reaction products of paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)” (MBO)” for life-cycle step production at Schülke & Mayr GmbH. S. Hahn, J. Regelmann, Fraunhofer Institute of Toxicology and Experimental Medicine, Department Chemical Risk Assessment, 24.7.2007 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.10/02	2007	Determination of total aldehyde in the waste water stream of Schülke & Mayr GmbH. Schülke & Mayr GmbH, Dr. Susanne Hendrich, 2.7.2007 (unpublished) non GLP, unpublished	Y	Schülke & Mayr
A2.10/03	2007	Estimation of the Environmental Concentrations and the Preliminary Environmental Risk Assessment of “reaction products of paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)” (MBO)” for life-cycle step production at Lubrizol Deutschland GmbH. S. Hahn, J. Regelmann, Fraunhofer Institute of Toxicology and Experimental Medicine, Department Chemical Risk Assessment, 24.7.2007 GLP not applicable, unpublished	Y	Lubrizol
A3.1.1/01	2001	Determination of the Melting Temperature / Freezing Temperature of 3,3’-Methylen-bis(5-methyloxazolidine) according to EC Council Directive 92/69/EEC, A.1. and OECD Guideline No. 102. BioChem Labor für biologische und chemische Analytik GmbH, Study No. 01 50 40 825 A, Sep. 20, 2001 GLP, unpublished	Y	Lubrizol

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A3.1.1/02	2001	Melting Point of GrotaMAR 71. Jai Research Foundation, Study No. 2660, Feb. 20, 2001 GLP, unpublished	Y	Schülke & Mayr
A3.1.2/01	2001	Determination of the Boiling Temperature of 3,3’-Methylen-bis(5-methyloxazolidine) according to EC Council Directive 92/69/EEC, A.2. and OECD Guideline No. 103. BioChem Labor für biologische und chemische Analytik GmbH, Study No. 01 50 40 825 B, Sep. 20, 2001 GLP, unpublished	Y	Lubrizol
A3.1.2/02	2000	Boiling temperature of GrotaMAR 71. Jai Research Foundation, Study No. 2661, Aug. 05, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.1.3/01	2000	Relative Density of GrotaMAR 71. Jai Research Foundation, Study No. 2662, July 27, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.1.3/02	2007	Determination of the Density of CONTRAM™ MBO. Lubrizol Industrial Additives, Hamburg July 4, 2007 No GLP, unpublished	Y	Lubrizol
A3.2/01	2000	Vapour Pressure of GrotaMAR 71. Jai Research Foundation, Study No. 2663, Nov. 07, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.2/02	2001	3,3’-Methylen-bis(5-methyloxazolidin), 24773, Vapour Pressure. Siemens Axiva Labor Sicherheitstechnik, Rep. No. 20011076.01, Nov. 02, 2001 GLP, unpublished	Y	Lubrizol
A3.2/03	2005	EPIWIN 3.12 estimation for 3,3’-Methylene-bis [5-methyl-oxazolidine] GLP not applicable, published	N	Not applicable
A3.2.1	2005	EPIWIN 3.12 estimation for 3,3’-Methylene-bis [5-methyl-oxazolidine] GLP not applicable, published	N	Not applicable
A3.4/01 IR	2007	Determination of the Infrared (IR) Spectrum of CONTRAM™ MBO. Lubrizol Industrial Additives, Hamburg July 4, 2007 No GLP, unpublished	Y	Lubrizol
A3.4/02 IR	2007	IR-Spectrum of Grotan OX Schülke & Mayr GmbH, Dr. S. Hendrich, 14.12.2007 No GLP, unpublished	Y	Schülke & Mayr
A3.4/03 MS	2007	Mass spectrum of Contram MBO Weber, L. University of Bielefeld, 09.07.2007	Y	Lubrizol

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		No GLP, unpublished		
A3.4/01 NMR	2004	Spektroskopische Untersuchungen zum Produktvergleich Contram MBO/ GrotaMar 71 Fraunhofer ITEM (Dr A Preiss), Draft Report, 9/04 No GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A3.4/02 NMR/MS	2002	Analysenbericht SMN9701, Formaldehyd/Aminopropanol Kondensate – Aufkärung der Struktur. Spectral Service, 15.März 2002 No GLP, unpublished	Y	Schülke & Mayr
A3.4/01 UV/VIS	2007	UV Spectrum of CONTRAM™ MBO Oxazolidine, 3,3'-Methylenebis [5-methyl]- (CAS# 66204-44-2) Lubrizol Metalworking Additives, Spartanburg, SC, USA, July 3, 2007 No GLP, unpublished	Y	Lubrizol
A3.4/ 02 UV/VIS	2007	UV/VIS Scan of GrotaMar 71 Schülke & Mayr Analytical Service, 18.6.2007 No GLP, unpublished	Y	Schülke & Mayr
A3.5/01	2002	Determination of the Water Solubility of 3,3’-Methylen-bis-[5-methyl-oxazolidine] according to OECD Test Guideline 105, flask method. Fraunhofer IME, Study No. BOD-003/7-14, Dec. 04, 2002 GLP, unpublished	Y	Lubrizol
A3.5/02	2000	Water Solubility of GrotaMAR 71. Jai Research Foundation, Study No. 2665, Sep. 14, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.7/01	2001	Fat Solubility of GrotaMAR 71. Jai Research Foundation, Study No. 2666, Oct. 15, 2001 GLP, unpublished	Y	Schülke & Mayr
A3.7/02	2007	Solubility of CONTRAM™ MBO Oxazolidine, 3,3'-Methylenebis [5-methyl]- (CAS# 66204-44-2) in Various Organic Solvents. Lubrizol Metalworking Additives, Spartanburg, SC, USA, July 2, 2007 No GLP, unpublished	Y	Lubrizol
A3.7/03	2006	Determination of the Solubility Range of CONTRAM™ MBO: Oxazolidine, 3,3'-methylenebis [5-methyl-], (CAS# 66204-44-2)) in n-Heptane Using a Turbidimetric Method. Lubrizol Metalworking Additives, January 13, 2006 No GLP, unpublished	Y	Lubrizol

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A3.9/01	2001	Partition Co-Efficient (n-Octanol/Water) of GrotaMAR 71. Jai Research Foundation, Study No. 2667, Aug. 06, 2001 GLP, unpublished	Y	Schülke & Mayr
A3.9/02	2002	Partition Coefficient (n-Octanol/Water) of GrotaMAR 71. Jai Research Foundation, Study No. 3603, Jan. 12, 2002 GLP, unpublished	Y	Schülke & Mayr
A3.9/03	2002	Determination of the Partition Coefficient n-octanol/water of 3,3’-Methylen-bis-[5-methyl-oxazolidine] according to OECD Test Guideline 117. Fraunhofer IME, Study No. BOD-003/7-07, Dec. 05, 2002 GLP, unpublished	Y	Lubrizol
A3.10/01	2003	Thermische Stabilität von MAR 71: Ergebnisse der DSC-Screeningtests Inburex Consulting, 11.März 2003 No GLP, unpublished	Y	Schülke & Mayr
A3.10/02	2007	Safety-related evaluation of the thermal stability of “CONTRAM(TM) MBO BC 6120 / 100495595”. Siemens AG, A&D AS SP PPE IPD Prozess-Sicherheit, Rep. No. PS 20070681-1-Kra No GLP, unpublished	Y	Lubrizol
A3.11/01	2000	Flammability of GrotaMAR 71. Jai Research Foundation, Study No. 2669, Aug. 04, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.11/02	2001	Auto-Ignition Temperature of GrotaMAR 71. Jai Research Foundation, Study No. 2670, Apr. 06, 2001 GLP, unpublished	Y	Schülke & Mayr
A3.12	2000	Flash Point of GrotaMAR 71. Jai Research Foundation, Study No. 2668, Aug. 07, 2000 GLP, unpublished	Y Y	Schülke & Mayr
A3.13	2007	Grotan OX, Surface Tension A.5. (OECD 115). Siemens Prozess-Sicherheit, Rep. No. 20070596.01, July 02, 2007 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A3.14	2007	Viscosity of Grotan OX Analytical Laboratory Schülke & Mayt GmbH, Dr. S. Hendrich, 6.12.2007 No GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A3.16	2000	Oxidation Property of GrotaMAR 71. Jai Research Foundation, Study No. 2671, Aug. 04, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.17	2007	Reactivity towards container material: CONTRAM™ MBO. Michael P. Scholz, Lubrizol, 19.07.2007 GLP not applicable, unpublished	Y	Lubrizol

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A4.1/02	2007	Analytical method of determination the content of releasable formaldehyde of GrotaMar 71 Schülke & Mayr GmbH, G.-D. Lembke, 18.12.2007 No GLP, unpublished	Y	Schülke & Mayr
A4.2b	2008	Statement on the Vapour pressure of “3,3' methylenebis[5-methyloxazolidine] (MBO): reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)”. Dr.Stefan Hahn, Fraunhofer ITEM, 20 February 2008 GLP not applicable, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.1	2000	Acute oral toxicity of Grotan WS in rats. Jai Research Foundation, JRF Study No. 2629 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.2/01	2002	Acute dermal toxicity test of “Contram 121” in the rat. Harlan Bioservice for Science, Study No. 10-4-0167-01 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.2/02	2000	Acute dermal toxicity of Grotan WS in rats. Jai Research Foundation, JRF Study No. 2630 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.4/01	2002	Acute dermal irritation/corrosion test of “Contram 121”in the rabbit. Harlan Bioservice, Study No. 10-3-0168-01 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.4/02	2000	Acute dermal irritation study of Grotan WS in the rabbit. Jai Research Foundation, JRF Study No. 2631 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.4/03	2000	Acute eye irritation study of Grotan WS in rabbits. Jai Research Foundation, JRF Study No. 2632 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.5/01	2001	OS157338, Skin sensitisation to the guinea-pig (Magnusson & Kligman method). Huntingdon Life Science Lt., Report No. LBL 045/004131/SS GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.5/02	2001	Skin sensitisation study of Grotan WS in guinea pigs (guinea pig maximisation test). JAI Research Foundation., Study No. 2633 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.3.1/01	2002	14-Day oral dose range finding toxicity study with “Contram 121” in the rat. Harlan Bioservice for Science, Study No.	Y (Exist./First)	Schülke & Mayr +

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		20-4-0155-01-01 GLP, unpublished		Lubrizol
A6.3.1/02	2002	Repeated dose 90-day oral toxicity study of Grotan WS in rats. JAI Research Foundation, India, Study No. 2636 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.4.1/01	2002	90-day repeated dose oral (gavage) toxicity study of “Contram 121” in the rat. Harlan Bioservice for Science, Study No. 20-4-0155-01 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.4.1/02	2002	Repeated dose 90-day oral toxicity study of Grotan WS in rats. JAI Research Foundation, India, Study No. 2636 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.1/01	2000	Salmonella typhimurium reverse mutation assay of Grotan WS. JAI Research Foundation, Study No. 2635 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.1/02	2000	OS157338: Reverse mutation assay “Ames test” using Salmonella typhymurium and Escherichia coli. Safepharm Laboratories, SPL Project No. 525/305 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.2	2001	OS157338: Chromosome aberration test in CHL cells in vitro. SafePharm Laboratories Ltd., SPL Project No. 525/303, draft GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.3/01	2001	OS157338: L5178 TK+/- mouse lymphoma assay. SafePharm Laboratories Ltd., SPL Project No. 525/304 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.3/02	2002	Grotan WS: L5178 TK+/- mouse lymphoma assay. SafePharm Laboratories Ltd., SPL Project No. 1598/002 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.4/01	2002	Mammalian micronucleus test of murine bone marrow cells with Contram 121. Bioservice Scientific Laboratories GmbH, Project No. 020225 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.6.4/02	2002	Mammalian bone marrow chromosome aberration test with Contram 121. Bioservice Scientific Laboratories GmbH, Project No. 011643	Y (Exist./First)	Schülke & Mayr + Lubrizol

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		GLP, unpublished		
A6.6.4/03	2000	Chromosomal aberration study of Grotan WS in mice. Jai Research Foundation, JRF Study No. 2634 GLP, unpublished	Y (Exist./First)	Schülke & Mayr + Lubrizol
A6.1.1/01	1977	Prüfung der akuten Toxizität von FO-I VP 1262, MK-ÄI2P an Ratten bei peroraler Verabreichung. Leuschner, F., Laboratorium für Pharmakologie und Toxikologie, 14. Sept. 1977 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.1/02	1979	Prüfung der akuten Toxizität von N,N-Methylen-bis(5-methyloxazolidin) an Sprague-Dawley Ratten bei oraler Verabreichung. Leuschner F., Laboratorium für Pharmakologie und Toxikologie, 8. Aug. 1979 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.1/03	2002	Acute toxicity study of 3,3'-methylenebis[5-methyl- oxazolidine] by oral administration to Sprague-Dawley rats. LPT Laboratory of Pharmacology and Toxicology, Report No. 14816/01 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.2/01	1977	Prüfung der akuten dermalen Toxizität von FO-I VP 1262, MK-ÄI2P – kurz „FO-I VP 1262“ genannt - an der intakten und skarifizierten Rückenhaut von Sprague-Dawley Ratten. Leuschner F., Laboratorium für Pharmakologie und Toxikologie, 26. Jan. 1977 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.2/02	2000	Acute dermal toxicity of GrotaMAR 71 in rats. Jai Research Foundation, Report No. 2623 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.2/03	2002	Acute toxicity study of 3,3'-methylenebis[5-methyl- oxazolidine] in Sprague-Dawley rats by dermal administration. LPT Laboratory of Pharmacology and Toxicology, Report No. 14817/01 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.4/01	1976	Test of the local and general tolerability of N,N-Methylene-bis(5-methyloxazolidine) in NZW rabbits (Patch test). Leuschner, F., Laboratorium für Pharmakologie und Toxikologie, 21st. Sept. 1976 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol

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A6.1.4/02	1979	Grotan OX, irritation studies in the rabbit (Part A, Draize skin irritation test). Consultox Laboratories Lt., Report CL79:187:1939 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.4/03	2002	3,3'-methylenebisoxazolidin/CAS-Nr. 66204-44-2. Primary skin irritation study in the rabbit. FREY-TOX, Lab. No. 02073 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.4/04	1979	Grotan OX, irritation studies in the rabbit (Part B, Modiefied Draize eye irritation test). Consultox Laboratories Lt., Report CL79:187:1939 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.4/05	1978	Draize eye test on ABt. FO-IL VP 1262. Standard Biological Laboratories, 25th Jan. 1978 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.5/01	2001	OS157339, Skin sensitisation to the guinea-pig (Magnusson & Kligman method). Huntingdon Life Science Lt., Report No. LBL 046/004056/SS GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.5/02	2001	Skin sensitisation study of Grotamar71 in guinea pigs (guinea pig maximisation test). JAI Research Foundation., Study No. 2624 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.5/03	1984	Guinea pig maximation tests with formaldehyde releasers. Results from two laboratories. Contact Dermatitis, 10: 257-266 Non-GLP, published	No	-
A6.3.1/01	2001	Repeated dose 90-day oral toxicity study of Grotamar71 in rats. JAI Research Foundation, India, Study No. 2628 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.3.1/02	2002	4-Week dose-range finding study for a 90-Day subchronic toxicity study of 3,3'-methylenebis[5-methyl- oxazolidine] by repeated oral administration to Sprague-Dawley rats. LPT Laboratory of Pharmacology and Toxicology KG, Report No. 14814/01 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.4.1/01	2001	Repeated dose 90-day oral toxicity study of Grotamar71 in rats. JAI Research Foundation, India, Study No.	Y	Schülke & Mayr + Lubrizol

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		2628 GLP, unpublished		
A6.4.1/02	2002	90-Day subchronic toxicity study of 3,3'-methylenebis[5-methyl-oxazolidine] by repeated oral administration to Sprague-Dawley rats. LPT Laboratory of Pharmacology and Toxicology KG, Report No. 14815/01 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.1/01	1997	Salmonella typhimurium reverse mutation test with MAR 71, Toxlabs®Prüflabor GmbH, Study No. Toxlabs®/1997/6926 RM GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.1/02	2000	Salmonella typhimurium reverse mutation assay of Grotamar 71. JAI Research Foundation, Study No. 2626 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.1/03	2000	OS157339: Reverse mutation assay “Ames test” using Salmonella typhimurium and Escherichia coli. Safepharm Laboratories Lt., Derby DE1 2BT, UK, Study 525/308 Non-GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.2	2001	OS157339: Chromosome aberration test in CHL cells in vitro. SafePharm Laboratories Ltd., SPL Project No. 525/306 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.3/01	2002	Grotamar71: L5178Y TK+/- mouse lymphoma assay. SafePharm Laboratories Ltd., SPL Project No. 1598/001 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.3/02	2001	OS157339: L5178 TK+/- mouse lymphoma assay. SafePharm Laboratories Ltd., SPL Project No. 525/307 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.4/01	2000	Chromosomal aberration study of Grotamr71 in mice. Jai Research Foundation, JRF Study No. 2625 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.6.4/02	2002	Micronucleus test of 3,3'-methylenebis[5-methyl- oxazolidine] in bone marrow of the NMRI mouse by oral administration. LPT Laboratory of Pharmacology and Toxicology KG, Report No. 14821/01 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.8.1	2006	Prenatal developmental toxicity study of	Y	Schülke &

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		N,N'-methylene-bis(5-methyloxazolidine) MBO in rabbits by oral administration. LPT Laboratory of Pharmacology and Toxicology GmbH & Co KG, Report No. 19547/05 GLP, unpublished	(Exist./First	Mayr + Lubrizol
A6.8.2	2007	N,N’-Methylene-bis-oxazolidine (MBO): Preliminary Reproduction Toxicity Study in the Han Wistar Rat. Study Plan. RCC Study Number B50927 & RCC Study Number B50916 Study Plan, unpublished	Y	Schülke & Mayr + Lubrizol
A6.12/01	1997	Kontaktallergien durch formaldehydab-spaltende Biozide. Allergologie, 20: 215-224 Non-GLP, published	No	-
A6.12/02	1998	Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. Brit J Dermatol, 138: 467-476 Non-GLP, published	No	-
A6.12/03	2002	Patch test reactions to Biobans in metal workers are often weak and not reproducible. Contact Dermatitis, 47: 27-31 Non-GLP, published	No	-
A7.1.1.1.1	2008	Hydrolysis of the equilibrium mixture of hexahydro-1,3,5-tris(2.hydroxypropyl)-s-triazine and N,N-methylene-bis-(5-methyloxazolidine) Fraunhofer ITEM (Dr A Preiss), Report 25.2.08 non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.1.1.2.1/01	2002	Study on the “Ready Biodegradability” of “3,3’-Methylenbis(5-methyloxazolidin)” according to OECD-Test Guideline 301D in the version of July 17th, 1992 (Closed-Bottle-Test). Institut Fresenius, Study No. IF-101/35291-00 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.1.1.2.1/02	2001	Ready Biodegradability of Grota MAR 71. Jai Research Foundation, Study No. 2652, Dec. 18, 2001 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.1.1.2.3	1996	A Study of the Aerobic Biodegradation in Seawater of MAR 71 using the Closed Bottle Procedure. Acer Environmental Laboratories and Sciences, Study No. AE950218 GLP, unpublished	Y	Schülke & Mayr + Lubrizol

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A7.1.3	2002	Estimation of the Adsorption Coefficient of 3,3’-Methylen-bis-[5-methyl-oxazolidine] according to Draft OECD Test Guideline 121. Fraunhofer IME, Study No. BOD-003/7-70, Dec. 05, 2002 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.3.1	2005	EPIWIN 3.12 estimation for 3,3’-Methylene-bis [5-methyl-oxazolidine] No GLP, published	N	Not applicable
A7.4.1.1/01	1995	Acute Toxicity Testing of MAR 71 in Zebra-fish (*Brachydanio rerio*) (Teleostei, Cyprinidae) under Semi-static Conditions. Arbeitsgemeinschaft GAB Biotechnologie GmbH und IFU Umweltanalytik GmbH, Report No. 95103/01-AABr GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.1/02	2000	Study on the Acute Toxicity Towards Fish of “3,3’- Methylene-bis [5-methyl-oxazolidine]” according to OECD-Test Guideline 203, Edition dated July 17th, 1992. Institut Fresenius, Study No. IF-99/21382-00 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.1/03	1997	Assessment of the aquatic-phase toxicity of MAR 71 to the marine fish *Scopthalmus maximus*. Environment & Resource Technology Ltd, Study No. 166-5-1 Non GLP, unpublished	Y	Schülke & Mayr
A7.4.1.2/01	2000	Study on the Acute Toxicity towards Daphnia of “3,3’- Methylene-bis [5-methyl-oxazolidine]” according to OECD-Test Guideline 202, Part I (“Daphnia sp., Acute Immobilisation Test”). Institut Fresenius, Study No. IF-99/21381-00 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.2/02	1995	Assessment of Toxic Effects of MAR 71 on *Daphnia magna* Using the 48 h Acute Immobilisation Test. Arbeitsgemeinschaft GAB Biotechnologie GmbH und IFU Umweltanalytik GmbH, Report No. 95103/01-AADm GLP, unpublished	Y	Schülke & Mayr + Lubrizol
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9 ANNEXES

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