

Committee for Risk Assessment
RAC

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

**Reaction products of paraformaldehyde and 2-
hydroxypropylamine (ratio 3:2); [MBO]**

EC Number: -
CAS Number: -

CLH-O-0000001412-86-95/F

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

Adopted
4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

**Substance Name: 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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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. 1272/2008², 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

**ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

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	<p>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</p> <p>Specific Conc. Limits: * Skin Corr.1B; H314: $C \geq 25 \%$ Skin Irrit. 2; H315: $5 \% \leq C < 25 \%$ Eye Irrit. 2; H319: $5 \% \leq C < 25 \%$ STOT SE 3; H335: $C \geq 5 \%$ Skin Sens. 1; H317: $C \geq 0.2 \%$</p>
2-Hydroxypropylamine	
Current entry in Annex VI, CLP Regulation	Skin Corr. 1B, H314: Causes severe skin burns and eye damage

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

**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

**ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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 $\leq 1\%$ and challenge response rates of $\geq 60\%$.

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)C₅₀ values between 1 - 100 mg/L; lowest acute value E_rC₅₀ (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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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

RAC general comment

The substance is an biocidal active substance which has originally been notified under the name "3,3'-methylene-bis(5-methyl-oxazolidine)" (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.

One of the by-products is α,α',α'' -Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) which is formed in the course of hydrolysis and is further hydrolysed to 2-hydroxypropylamine and formaldehyde. The active substance notified as MBO was renamed to "*reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)*" further referred to throughout this opinion as "*RP 3:2*".

For several endpoints data on RP 3:2 as well as the hydrolysis products formaldehyde and 2-hydroxypropylamine were also considered.

According to the CLH report, the closely related substance RP 1:1 (see RAC opinion on *reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)*), contains about 28% (calculated) releasable formaldehyde while RP 3:2 contains about 45% (calculated) releasable formaldehyde per molecule. However, IND have argued (during public consultation) that 'free' (unbound) residual formaldehyde is present at very low concentrations.

From the data in the "Environmental hazard assessment" section of the CLH report, it can be concluded that substantial amounts of formaldehyde (or formaldehyde hydrate) can be released from RP 3:2 within 1 h at physiological pH. However, the extent of hydrolysis of RP 3:2 (and therefore release of formaldehyde from the substance) is highly dependent on the extent of dilution with water. As shown in Table 5.1.1.-3 of the CLH

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

report, at a 1% (w/w) initial concentration of RP 3:2 (MBO; Grotan® WS), in a buffered aqueous solution at pH= 7 (at 25°C), the formaldehyde content reached 27% of the constituents within 1 h. The total concentration in the solution was very low, due to the low starting material concentration), and the formaldehyde formation rate appearing to plateau after this point. Separately it was shown that in an unbuffered solution of D₂O, at a 1% initial concentration of MBO (RP 3:2; Contram™ i.e apparently from another source) (at 20°C), formaldehyde hydrate content reached approximately 10% of the constituents (but only a low concentration in the solution, again due to the low concentration of starting material), but the time at which the sampling occurred was not stated (see table 5.1.1.-2 of the CLH report). Since the dossier submitter (DS) noted in the response to comments (RCOM) that the test material had a pH of around 9.0-10.0, the data seemed consistent with the findings in Table 5.1.1.-3, where in a buffered solution at pH = 9 the formaldehyde content reached approximately 7% after 33 min and 9% after 6.9 h. The highest degree of formaldehyde formation was seen at pH 4 (38% within 0.33 h, 42% within 6.8 h). For further details see the discussion under "RAC evaluation of environmental hazards", Section 5.1.1 of the CLH report and the RCOM.

Available hydrolysis tests support qualitatively that hydrolysis will occur in contact with aqueous biological media in mucous membranes. Inhalation exposure to aerosolic RP 1:1 is expected to result in hydrolysis at the site of contact and toxicologically significant concentrations of formaldehyde could be reached on the surface of the mucous membranes in the respiratory tract, eye or upper GI tract or skin. The inhalation exposure to gaseous formaldehyde evaporating from RP 1:1 is assumed to contribute to the toxic/carcinogenic effect resulting from the direct impact of hydrolysis products at the contact site.

The CLP Regulation, Art. 9 and Annex 1, 1.1.1.3, supports a weight of evidence evaluation of the available data. Where data on RP 3:2 are lacking, data on RP 1:1 and data on the hydrolysis products formaldehyde and 2-hydroxypropylamine were therefore considered. Regarding the quality of the available data and the consistency of information on the toxic effects and the related mode of action, information on hazardous properties on these 'source' substances are in general considered appropriate to predict the hazardous properties of RP 3:2.

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.

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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 ^1H 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 formaldehyde which can be released from MBO (correctly described as RP 3:2) in one of the following ways:

	Molar mass [g]	% referred to MBO ^(*)	% referred to MBO + 3 mol water
2 mol 2-hydroxypropylamine	150	80,6	62,5
3 mol Formaldehyde	90	48,4	37,5
<i>Sum</i>	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.

**ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

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

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PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

	N v3.10			
Physical state	Visual inspection	n.a.	liquid	Doc. III-A 3; A3.1.1/01 and Study A3.1.1/02
Colour	Visual inspection	n.a.	colourless to yellowish	Doc. III-A 3; A3.1.1/01 and 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	¹ H-NMR ¹³ C-NMR	CONTRAM™ MBO and GrotaMAR 71; Charge 1057472	¹ H- NMR spectrum and ¹³ C- NMR spectrum is consistent with the proposed structure of MBO.	Doc. III-A 3; Study A 3.4/01 NMR
	¹ H-NMR ¹³ C-NMR	MAR 71; Charge 1047946	¹ H- NMR spectrum and ¹³ C- 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 massspectrometer	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;

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PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

	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 flask 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 flask 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

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			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 $\sigma=68.1$ 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 algacides 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

Slimeicides, product type 12

Metal-working fluid, product type 13

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PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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

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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.

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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 CO ₂ 2) Direct enzymatic conversion to formate and utilisation for C1-transfer or oxidation to CO ₂ 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 CO ₂ 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 t _{1/2} 12 h, terminal t _{1/2} 50 h, 10-40 % ¹⁴ C residues after 3-4 d)		

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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 aqueous media.

For formaldehyde 100% absorption via all routes 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 LD ₅₀	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), DocIII A6.1.1
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), DocIII A6.1.2/01
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), DocIII A6.1.2/02

f: females; m: males

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

The acute toxicity after oral and dermal exposure has been investigated in valid studies on experimental animals. The oral LD₅₀ in rats is 960 mg/kg bw. Primarily local effects in the gastro-intestinal tract were observed (**cf. DocIII A6.1.1**). 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. DocIII A6.1.2/01**).

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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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
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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/kg 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.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS included data on reaction products of paraformaldehyde with 2-hydroxypropylamine for both RP 1:1 and RP 3:2. For RP 1:1 there is one oral rat study and two dermal rat studies. The LD₅₀ were 960 mg/kg bw for acute oral toxicity and above 2000 mg/kg bw for acute dermal toxicity. For RP 3:2 there were three oral rat studies and three dermal rat studies. The LD₅₀ were 630 mg/kg bw for acute oral toxicity and 790 mg/kg for acute dermal toxicity. No data was available on inhalation toxicity.

Although the data could be considered to support classification for acute toxicity, the DS stated that the effects were due to the corrosive effect of the substance and therefore

proposed no classification for acute toxicity.

Comments received during public consultation

Two MSCAs suggested that classification for acute toxicity may be applicable. One MSCA also proposed addition of the supplemental hazard information statement EUH071 (corrosive to the respiratory tract) and raised the possibility of adding EUH029 (contact with water liberates toxic gas).

Assessment and comparison with the classification criteria

Acute oral toxicity

RP 1:1

Local effects in the GI tract were seen in the acute oral toxicity study on RP 1:1 (Schülke & Mayr, 2000). However, its severity, dose-response relationship and whether it contributed to the mortality remains unclear. The LD₅₀ value for RP 1:1 was 960 mg/kg bw.

Formaldehyde

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

2-Hydroxypropylamine

In the document CLH-REP_ATT_Appendix HPA_DV018252-32 (hereafter referred to as "Doc Appendix HPA") two studies were cited revealing an LD₅₀ of 4260 mg/kg bw (Smyth *et al.*, 1949) and an LD₅₀ of 2100 mg/kg bw (Carreon & Yakel, 1981).

There is no harmonised classification for 2-Hydroxypropylamine for this endpoint. Classification as Acute Tox. 4; H302 - Harmful if swallowed is notified in the C&L Inventory.

RP 3:2

The oral LD₅₀ of 630 mg/kg bw was derived from a study according to OECD technical guideline (TG) 423 where mortality at 2000 mg/kg bw was 100% and no effects were seen at 200 mg/kg bw (Bode Chemie, 2002). Local effects along the upper GI tract that could indicate corrosivity of the 10% test solution in corn oil were not reported for this study. Also, two further oral studies (Schülke & May, 1977, 1979) did not show local effects at test concentrations of 8% and 10%, respectively, in aqueous 0.9% NaCl solution. The DS explicitly noted that surprisingly no local effects were detected in the oral studies. In both studies, the clinical effects in surviving rats were reversible within 24 hours.

Concerning the DS proposal not to classify for acute oral toxicity due to the classification as a corrosive substance, RAC does not find a general disclaimer on acute toxicity for non-classification of corrosive substances in the CLP Regulation. In addition, there are no data that clearly indicate that the mechanism of toxicity was corrosivity.

In conclusion, an OECD TG 423 (acute toxic class method) study on RP 3:2 revealed an LD₅₀ value of 630 mg/kg bw and two other studies with a test design similar to OECD TG 401 resulted in oral LD₅₀ values of 750 and 900 mg/kg bw. Thus, RAC agrees to classify RP 3:2 (based on the studies on RP 3:2) as **Acute Tox. 4; H302 (Harmful if swallowed)** according to the CLP Regulation (ATE guidance values for this category are from > 300 to ≤ 2000 mg/kg bw).

Acute dermal toxicity

RP 1:1

Two acute dermal studies on RP 1:1 revealed LD₅₀ values > 2000 mg/kg bw.

Formaldehyde

Formaldehyde has a harmonised classification in CLP, Annex VI for Acute dermal toxicity, category 3; H311 (Toxic in contact with skin).

2-Hydroxypropylamine

In Doc Appendix HPA, an LD₅₀ of 1640 mg/kg bw was calculated in the study of Smyth *et al.* (1949) and the study of Carreon & Yakel (1981) identified an LD₅₀ of 1850 mg/kg bw, both in rabbits.

There is no entry in CLP, Annex VI for Acute dermal toxicity. Some self-classifications as Acute Tox. 4; H312 (Harmful in contact with skin) are available.

RP 3:2

Corrosivity was not observed after dermal application of undiluted test substance in two studies on RP 3:2 with a comparable design to OECD TG 402. Slight skin reddening (Draize score 1, 10-24 h) seen at ≥ 5.04 mL/kg was obviously not the cause of death in the study of Schülke & Mayr (1977) with LD₅₀ at 6000 mg/kg bw. Local erythema (6 h post application and local scale formation at study termination (14 days post exposure) were observed in another study of Schülke & Mayr (2000). This study revealed an LD₅₀ of 1400 mg/kg bw. At study termination (14 days post exposure) skin histopathology of surviving rats at ≥ 1000 mg/kg bw showed (non-reversible) effects such as local cellular debris and necrotic tissue (scab), leucocyte infiltration and epidermal cyst. However, no information could be found on the severity and extension of the skin lesions. Necrotic tissue (scab formation) was only reported for the survivors at study termination and was likely not the cause of mortalities.

The lowest LD₅₀ of 760 mg/kg bw was estimated for female rats (males and females combined LD₅₀ 790 mg/kg bw) (Bode Chemie, 2002). No local skin effects were observed in all animals at this dose. At 2000 mg/kg bw (test substance was undiluted at this dose), 1/5 males had erythema and slight oedema (5/5 males died on day 1-7), 4/5 females had slight to severe erythema and slight to severe oedema (5/5 females died on day 1-7). As no indication on skin necrosis and scab formation was reported in only 2/5 female animals at 2000 mg/kg bw and 4/5 males died without any skin effects, the mortalities observed can not be explained by corrosive effects.

The DSproposed classification for Acute dermal toxicity, category 4; H311 as appropriate in the CLH report. Based on the DS's interpretation that the effects are secondary to corrosivity, the final proposal was not to classify for Acute dermal toxicity.

RAC agrees that based on the lowest acute dermal LD₅₀ value of 760 mg/kg bw in female rats, RP 3:2 should be classified as **Acute Tox. 3; H311 (Toxic in contact with skin)** according to CLP (dermal ATE guidance values for this category are from > 200 to ≤ 1000 mg/kg bw). As mortalities occurred without local skin effects or scab formation due to necrotic precursor lesions were seen in the surviving animals at the end of the 14-day observation time, skin lesions are unlikely to be the cause of mortalities.

Acute inhalation toxicity

RP 1:1

Studies on acute inhalation toxicity were not available on RP 1:1.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

Formaldehyde

There are acute inhalation studies (see Formaldehyde Core Document) suggesting that corrosive effects in the upper respiratory effects may contribute (possibly in addition to other effects) to lethality: histopathological examination revealed excessive mucus secretion, mucociliary dysfunction, single cell necrosis, and discontinuous nasal epithelium with erythrocyte leakage following 4 h of exposure of rats to formaldehyde gas concentrations of 12 µg/L (Bhalla *et al.*, 1991). Higher concentrations (0.6-1.7 mg/L) resulted in haemorrhage and oedema of the lung as well as oedema in liver and kidneys and hepatocyte necrosis (Skog, 1950). The Formaldehyde Core Document indicates an LC₅₀ of 0.6 mg/L (4 h).

Formaldehyde is classified in CLP, Annex VI as Acute inhalation toxicity, category 3; H331 (Toxic if inhaled).

2-Hydroxypropylamine

According to the information in Doc Appendix HPA, no mortality was found in rats exposed for 8 h to saturated vapour (Smyth *et al.*, 1949; post exposure observation period 14 days, no further data available). Twelve rats were exposed for 8 h to air saturated with 2-hydroxypropylamine at 20°C. No clinical symptoms were detected and no effects were seen at necropsy (no further details; BASF AG, 1965, cited in Greim, 1994).

There is no entry in CLP, Annex VI for Acute inhalation toxicity.

RP 3:2

Studies on acute inhalation toxicity were not available on RP 3:2.

During public consultation one MSCA suggested that read across to formaldehyde could be justified. This MSCA noted that the level of formaldehyde emission may not be constant over time and proposed classification on acute inhalation toxicity as Acute Tox. 4; H332 (Harmful if inhaled) for RP 3:2.

The CLP Guidance, 3.1.2.3.3 states that 'Corrosive substances (and mixtures) may be acutely toxic after inhalation to a varying degree and by different modes of action. Therefore, it is not possible to estimate the acute inhalation toxicity from the corrosivity data alone.

The DS considered Acute inhalation toxicity, category 4; H332 appropriate for RP 1:1 and RP 3:2 based on the read across from formaldehyde vapour to released mist with 28% formaldehyde content. RAC emphasises that the correct amount of releasable formaldehyde for RP 3:2 is 45%.

RAC considers that read across to formaldehyde is justified as RP 3:2 contains 45% releasable formaldehyde. Using the data supporting formaldehyde classification (Cat. 3) and taking the maximum amount of releasable formaldehyde into account, Acute Tox. 4 is considered as justified assuming that the total amount of 45% formaldehyde is indeed released. This is consistent with the observation that acute toxicity values for the oral route demonstrated lower potency of RP 3:2 than formaldehyde to cause acute toxic effects. As hydrolysis data in contact with biological tissues are lacking, RAC discussed the uncertainties with regards to the actual emitted concentrations in air (as gaseous phase or aqueous solution), and further uncertainties that may result from unstable intermediates which could also contribute to the acute inhalation toxicity. For RP 3:2 a LC₅₀ of about 1.3 mg/L (factor of 2.2 applied on a LC₅₀ of 0.6 mg/L (4h) for formaldehyde) would result. For mists, this is equivalent to Cat. 4 (ATE guidance values from >1 to ≤5 mg/L).

RAC, therefore agrees to classify RP 3:2 as Acute Tox. 4 (Harmful if inhaled).

EUH071

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

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

In line with previous RAC recommendations where EUH071 has been assigned in addition to the classification on acute inhalation toxicity, **RAC agrees to assign EUH071.**

EUH029

The labelling EUH029 (Contact with water liberates toxic gas) was suggested for consideration by one MS. CLP, Annex II, 1.2.1 defines that substances and mixtures which in contact with water or damp air, evolve gas classified for acute toxicity in category 1, 2 or 3 in potentially dangerous amounts should be labelled with this phrase.

RAC discussed that the liberation of toxic gas after contact with water will not be the main concern as sufficiently high amounts of toxic gas may not immediately be produced. Formaldehyde will also be generated and released without contact with water as aqueous conditions are given under normal room air conditions in contact with mucous membranes (of the eye, the respiratory tract and the upper GI tract) and in contact with sweaty skin.

It is also to note that the CLP, Annex 11, 1.2.1 foresees the additional labelling with EUH029 only for substances classified for Acute toxicity in category 1,2 or 3 and not for Acute Tox. 4 substances.

RAC concludes that EUH029 is not warranted.

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.

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

Summary of the Dossier submitter's proposal

The DS argued that STOT SE 3; H335 is not appropriate as the substance is corrosive.

Comments received during public consultation

Two MSCAs remarked that the classification STOT SE is not (generally) covered by the

classification Skin Corr. and one MSCA wished to consider data on formaldehyde for classification as STOT SE. One MSCA agreed that no classification for STOT SE 3 is required.

Assessment and comparison with the classification criteria

RP 1:1

There is no proposal to classify RP 1:1 for STOT SE 1, 2 or 3.

Formaldehyde

For formaldehyde, there is no entry in Annex VI on STOT SE; some notifiers self-classified for STOT SE.

2-Hydroxypropylamine

There is no entry in Annex VI on STOT SE. There is no robust information to judge on STOT SE.

RP 3:2

Based on the acute toxicity data on RP 3:2 there were no effects beyond those covered by the classifications on acute dermal and oral toxicity that would justify STOT SE 1 or 2.

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

'In general, a classification for corrosivity is considered to implicitly cover the potential to cause RTI and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed.'

Following the CLP criteria STOT SE 3 should also be considered as covered by Skin Corr. 1B.

RAC agrees with the DS that **no classification on STOT SE is warranted**, and that the potential for respiratory tract irritation is covered by the classification of RP 3:2 as corrosive to the skin.

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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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			
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

**ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF
PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]**

Species	Method	identity as given in study report	Score 1h, 24h, 48h, 72h / average score 24,48,72 h after patch removal		Reversibility	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. DocIII A6.1.4/01) 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 from 1976 the test substance was not applied directly to the skin. Schülke & Mayr (1979, cf. DocIII A6.1.4/02) 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 1:1 as well as RP 3:2 should be classified as Skin Corrosive Category 1, H314 - Causes severe skin burns and eye damage.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Two rabbit OECD TG 404 (or comparable) studies on RP 1:1 and three on RP 3:2 were summarised in the CLH report. The DS also included discussion on the hydrolysis products of the substance, formaldehyde and 2-hydroxy-propylamine. The results suggested strong irritant to corrosive properties. The DS found subcategorisation difficult based on the data, but, as Skin Corr. 1 without subcategorisation is not yet possible, proposed Skin Corr. 1B. In arriving at this decision more weight was put on the more recent studies.

Comments received during public consultation

Comments in agreement with the proposed classification for Skin corrosivity category 1 without subcategorisation were submitted by three MSCA.

Assessment and comparison with the classification criteria

RP 1:1

Irritant and corrosive properties were observed for RP 1:1 in two studies conducted according to OECD TG 404 (Becker Chemie, 2002, Schülke & Mayr, 2000). Exposure periods of 3 minutes and 1 hour were also tested in the study of Becker Chemie (2002) and these revealed only well defined erythema at 4 h postexposure.

Formaldehyde

Formaldehyde is classified in CLP, Annex VI as Skin Corr. 1B; H314 (Causes severe skin burns and eye damage).

2-Hydroxypropylamine

2-Hydroxypropylamine is classified in CLP, Annex VI as Skin Corr. 1B; (H314 Causes severe skin burns and eye damage).

RP 3:2

Skin corrosion and strong irritation was observed in two studies on RP 3:2, one of which (with 4 h exposure time) was in full compliance with OECD TG 404 (Bode Chemie, 2002). In this study brown discolouration, necrotic tissue, and demarcation was reported as non-reversible in 2/3 rabbits on day 7, on day 14, on day 21 (2 animals had granulation tissue, synonymous with scar formation following necrosis), and on day 28 (scarring was seen in 2 animals) (see study report in Doc III A6.1.4/03).

RAC notes that the DS proposed Skin Corr. 1 without a subcategory in chapter 4.4. of the CLH report and revised the proposal to Skin Corr. 1B following the response from one MSCA. The CLP Regulation does not foresee a classification for skin corrosion without subcategorisation. In contrast to the DS argumentation in the CLH report, it is RAC's view that the available data enable a decision to be made on the subcategory. The translation rules of Annex II may give general guidance (but does not reflect the quantitative aspects needed for subcategorisation), but more emphasis is given by RAC on the study information on RP 3:2 and studies on RP 1:1 and formaldehyde were taken as additional information.

Corrosive effects were seen in 2/3 animals in the study of Bode Chemie (2002). The earliest time point, when brown discolouration (interpreted as indicative for epidermal cell destruction as brown discolouration and necrotic tissues was reported from day 7 onwards for this and the second rabbit) was observed, was after 1 hour (of 4 h exposure time) in one animal. The same lesions were reported for the second rabbit beginning after 48 h. According to the CLP criteria (Annex I, Table 3.2.1) the subcategory 1B is appropriate when corrosive effects appeared during the 14 day observation time after exposure for > 3 min and ≤ 1 h. Although exposure periods of less than 4 h were not tested on RP 3:2, the first reading was done after 1 h in the study of Bode Chemie (2002). As corrosive effects in this study began after 1 hour exposure duration, RAC proposes to assign subcategory 1B based on the 1 h observation and taking read across to formaldehyde and 2-hydroxypropylamine into account.

Irritant and corrosive effects were also reported for all animals of the second study (Schülke & Mayr, 1979) using a 24 h exposure period. The report on the study results does not allow a subcategorisation due to the extended exposure period and limited readings (only at 24 and 72 h, see Doc III A6.1.4/02).

As RP 3:2 was tested for skin irritation/corrosion in undiluted form which should not contain relevant concentrations of formaldehyde or 2-hydroxypropylamine, the observation of corrosivity supports either that RP 3:2 itself has corrosive properties or that a sufficiently rate of hydrolysis will occur within a short period of exposure (within 1 h) that caused corrosive effects by the hydrolysis products (formaldehyde and 2-hydroxypropylamine).

Based on the study of Bode Chemie (2002) on RP 3:2 and the observation that the signs of corrosivity were already noticed at the first reading after 1 hour and read across to the corrosive hydrolysis products, **RAC agrees on the classification as Skin Corrosive 1B; H314 (Causes severe skin burns and eye damage).**

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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				Reversi- bility	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				Reversi- bility	Remarks/ results	Reference
			Cornea	Iris	Chemosis Conjunctiva	Redness Conjunctiva	Yes/No		
Rabbit	Compar- able 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 sacri- ficed	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 Pro- ducts (1978) DocIII A6.1.4/05

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

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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.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

For a skin corrosive substance eye irritation studies should normally not be conducted. However, a number of such studies were submitted by the applicant in the biocide process and these were summarised in the CLH report. One study with each substance (RP 1:1 and RP 3:2) was OECD TG 405 compliant (or comparable). In addition, one supportive non-guideline study was included.

The DS concluded that studies indicate that the substance is eye corrosive.

Comments received during public consultation

Comments in agreement with the DS proposal were submitted by one MSCA. It was pointed out by another MSCA that although no labelling is required as the substance is also skin corrosive the substance should be classified for Eye damage category 1.

Assessment and comparison with the classification criteria

RP 1:1

A guideline conforming acute eye irritation study on RP 1:1 resulted in irreversible cornea

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

lesions at day 21.

Formaldehyde

The Annex VI entry on formaldehyde do not include eye irritation/damage, however the majority of notifiers have self-classified the substance as Eye Dam. 1.

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

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

In humans, indications of eye irritation such as increased eye blink frequency and conjunctival redness were seen from gaseous concentrations of $600 \mu\text{g}/\text{m}^3$ (WHO 2010).

2-Hydroxypropylamine

Studies reporting corrosive properties to eyes were documented in Doc Appendix HPA.

There is no Annex VI entry covering eye irritation/damage on 2-hydroxypropylamine, but the majority of notifiers classify the substance as Eye Dam. 1.

RP 3:2

The DS noted that the irreversible eye damage would support classification for Eye damage category 1, but considered a separate classification as not required as the labelling for H314 (Causes severe skin burns and eye damage) already covers eye damage.

The study of Schülke & Mayr (1979) demonstrated severe effects (iris lesions after 24 hours, mean 2.0 (which means haemorrhage, gross destruction, or no reaction to light according to OECD TG 405) that were considered as irreversible. Animals were sacrificed due to the severity of ocular reactions 24 h after instillation.

The CLP guidance stipulates in section 3.3.2.4:

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

Also, the CLP Guidance in section 3.3.2.6 indicates in step 0 that:

"if the substance is classified as a skin corrosive, the substance is classified for serious eye damage but not labelled for serious eye damage."

However, the CLP guidance is not clear with regards to a separate classification for corrosive effects on the eye. The first sentence of CLP guidance section 3.3 recommends:

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

In previous cases of corrosive substances, RAC decided not to propose a separate classification for serious eye damage. For RP 3:2 RAC agrees to classify as Eye Dam. 1. Although for corrosive substances the risk for severe eye damage is implicit (and testing should not be avoided), in this case severe eye damage has been demonstrated in animal studies (conducted in 1978 and 1979) and **justifies a separate classification as Eye Dam. 1. Separate labelling with H318 is not needed.**

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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); DocIII A6.1.5/01
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); DocIII A6.1.5/02

For this endpoint one reliable study is available (see table above). In a Guinea pig maximisation test (GPMT, cf. DocIII A6.1.5/01) 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 (DocIII A6.1.5/02) 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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 3:2); [MBO]

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).

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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. DocIII A6.12/01). 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. DocIII A6.12/02) and Brinkmeier et al. (2002, cf. DocIII A6.12/03; 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).

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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.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS included five Guinea Pig Maximisation Test (GPMT) tests in the CLH report which were conducted in accordance with or were comparable to OECD TG 406; two studies used RP 1:1 and three used RP 3:2. For RP 1:1, one study resulted in > 60% sensitisation at 1 % induction concentration but it was concluded that the study was unreliable. For RP 3:2, one study with a very low intradermal induction dose (0.01%) was negative and two were positive. The effect rate was 60% at a 1% induction dose and > 90% at a 5% induction dose, respectively. In addition there is human data described for RP 3:2, in which 3.1% of 1786 patients showed sensitivity. The DS proposed to classify as Skin Sens. 1A; H317.

Comments received during public consultation

Two MSCAs submitted comments in agreement with the DS's proposal.

Assessment and comparison with the classification criteria

Animal data on RP 1:1

A GPMT study (Lubirzol Corporation, 2001) is available supporting Skin sensitisation category 1A due to a positive response to a 1% induction concentration in 90% of animals.

Formaldehyde

The existing classification of the hydrolysis product formaldehyde in Annex VI to CLP is Skin Sens. 1; H317.

2-Hydroxypropylamine

There is no evidence for sensitizing properties in available human studies (with limited documentation). The Doc Appendix HPA documented summaries on two patch test series in volunteers with 0.2 ml 2% aqueous solution of hydroxypropylamine as negative. A questionnaire to workers exposed to 2-hydroxypropylamine revealed that 5 of 15 randomly selected individuals reported contact dermatitis. This study was considered of limited validity, presumably due to the irritant effects observed after direct contact with 2-hydroxypropylamine.

RP 3:2

Human data on RP 3:2

In a study on contact allergy, numerous formaldehyde releasers were tested and among these the highest frequency was observed for RP 3:2 (Geier *et al.*, 1997, Doc III A6.12/01).

Epicutaneous testing in 1786 patients (from 35062 patients in 24 hospitals) exposed to RP 3:2 for 24 h or 48 h revealed allergic skin reactions in 55 patients (3.1%). In this study,

46 out of 1406 patients tested with formaldehyde and in separate experiments with RP

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3:2 showed positive reaction to RP 3:2. As only 13 out of these 46 patients were also positive to formaldehyde, it was concluded that the positive results might primarily result from the UVCB substance and not only from released formaldehyde.

DeGroot *et al.* (2010) reviewed five patch test studies on patients who were metal workers with suspected contact dermatitis, and who had contact with metal working fluids containing RP 3:2, and found positive reactions in 2.3% to 6.7% of the exposed workers (see Table 2 in this publication).

According to the CLP Guidance, 3.4.2.2.3 on the evaluation of human data, frequencies of 2.3% to 6.7% as reported for the metal workers correspond to the high frequency level (Table 3.4.2-b, the guidance level for high frequency in selected workers with known exposure or dermatitis is $\geq 1.0\%$) and supports a subcategorisation as Skin Sens. 1A.

Regarding the exposure level and its relevance for subcategorisation, no data were included in the CLH report to enable human exposure to be estimated for subcategorisation in accordance the CLP Guidance.

Animal data on RP 3:2

In the GPMT for RP 3:2, the intradermal induction dose of 0.5% resulted in 60% positive animals after challenge with a 1% solution (Anderson *et al.*, 1984).

A positive sensitisation response $> 90\%$ was observed in another GPMT study by Schülke & Mayr (2001). This study cannot be used for subcategorisation as a high induction dose (5%) was applied, and a decision concerning whether to classify in Cat. 1A or 1B, requires induction concentrations below 1% to have been tested.

The results of the Anderson *et al.* (1984) study meet the criteria for subcategory 1A ($\geq 60\%$ responding at $>0.1\%$ to $\leq 1\%$ intradermal induction dose). RAC concludes that the information from the animal testing is supportive of subcategory 1A.

RAC agrees with the DS's proposal to classify RP 3:2 as Skin Sens. 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 (gavage)	14 days; GLP	Rat Wistar 5 m & 5 f	0, 50, 100, 200 mg/kg bw; = 2, 4,	Contram 121 batch 24774	Body weight and food consumption ↓	200 mg/kg bw	100 mg/kg bw	Becker Chemie (2002); DocIII A6.3.1/01

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			8% in peanut oil; once daily, 7 days/week		at 200 mg/kg bw. Dose-range finding for 90d study;			
Oral (gavage)	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); DocIII A6.3.1/02
Oral (gavage)	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 6 % in peanut oil; once daily, 7days per week	Contram 121 batch 24774	≥ 80 mg/kg bw: clinical signs (breathing sounds), mortality, lesions of larynx and pharynx; 150 mg/kg bw: lesions of oesophagus in f	80 mg/kg bw	30 mg/kg bw	Lubrizol Deutschland GmbH (2002); DocIII A6.4.1/01
Oral (gavage)	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 conclusion)	40 mg/kg bw/day (authors conclusion)	Schülke & Mayr (2002); DocIII A6.4.1/02

In a 90-day gavage study according to OECD guideline 408 (cf. DocIII A6.4.1/01) 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. DocIII A6.4.1/02) 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.

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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

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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. DocIII A6.4.1/02) 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 granulocytes 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	Gavage (corn oil)	Gavage (corn oil)	Via drinking water
Target organs	Local effects larynx, pharynx & oesophagus	Mainly local effects Stomach	local effects
Study duration	90 days	90 days	2 years
Species	Rat	Rat	Rat
LOAEL in mg/kg bw/day	80 (LOAEC 3.2%)	60 (LOAEC 1.2%)	82 (m) or 109 (f) (0.19%)
NOAEL in mg/kg	30 (NOAEC 1.2%)	20 (NOAEC 0.4%)	15 (m) or 21 (f) (0.026%)

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bw/day			
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/m ³) NOAEC (mg/m ³)	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 LOAELs/LOAECs 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

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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 than mediating the evident acute toxicity”, the oral LD50 (see chapter 3.9.2.5.1 in ECHA CLP guidance 2012).

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

4.7.7 Conclusions on classification and labelling for STOT RE

No classification necessary for STOT RE is required.

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

Summary of the Dossier submitter's proposal

A number of oral studies in the rat were available for both RP 1:1 and RP 3:2. No dermal or inhalation studies were available. Although some effects are seen below the guidance values, these effects are concluded to be due to the corrosivity of the test compound and thus, according to the DS, no classification is warranted.

Comments received during public consultation

Two MSCA did not agree that STOT RE is covered by the classification for corrosion and asked for this classification to be considered or, if data are lacking, to refer to formaldehyde.

One MSCA proposed STOT RE 2, referring to the provision of section 3.9.2.5.1 of the CLP Regulation stating that if repeated dose effects occur at doses more than half an order of magnitude lower than the acute toxicity, classification for STOT RE should be considered.

In addition to comparing repeated oral toxicity with acute oral toxicity, the stomach lesions in rats treated with RP 3:2 in the OECD TG 415 study at 45 mg/kg bw over 70 days of treatment support classification as STOT RE.

Assessment and comparison with the classification criteria

Oral route

RP 1:1

The DS indicated the 90-day study of Lubrizol Deutschland GmbH (2002) to be of higher relevance than the second 90-day study of Schülke & Mayr (2002), both conducted in accordance to OECD TG 408.

In the first gavage 90-day study with 10 male and 10 female rats per dose group that received 0, 12, 30, 80 or 150 mg/kg bw/d RP 1:1 (concentrations 0, 0.48, 1.2, 3.2 or 6% in peanut oil) (Lubrizol Deutschland GmbH, 2002), 2 males died after the first dose of 200 mg/kg bw/d which was then reduced to 150 mg/kg bw/d. Lesions in this region were found at this dose in both males and in one male that died at day 52 and in one female

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that died at day 75.

Abnormal breathing sounds were noted in animals at 80 mg/kg bw/d at week 5 or later (in one female that died on day 68, 3 males, including one male which died on day 68 with pharyno-laryngeal lesions,) and 150 mg/kg bw/d (4 males, 3 females starting at week 2). From the latter dose, 2 males and 2 females showed poor general condition and reduced activity. Reduced motor activity was observed in one female and one male at 30 mg/kg bw/d and in one female at 150 mg/kg bw/d.

Histopathology on animals which died during the exposure period revealed laryngitis in 1/1 male at 80 mg/kg bw/d and in 2/3 males and 1/1 female at 150 mg/kg bw/d, ulcerative laryngitis in 1/3 males at 150 mg/kg bw/d and pharyngitis in 1/3 males at 150 mg/kg bw/d and oesophagus lesions (mural inflammation and myopathy) in 3/9 females and mural inflammation only in 1/9 females at 150 mg/kg bw/d. The summary table in the HPT Doc III A gives the impression that a microscopic examination on the larynx, pharynx and oesophagus was limited to the intermittent deaths.

In surviving animals at 150 mg/kg bw/d, purulent rhinitis was observed in 1/7 males and 1/9 females and stomach submucosal inflammation in 1/7 males.

No treatment-related findings were seen at 30 mg/kg bw/d except in one female that died on day 38 with reduced activity, reduced skin turgor, reduction of bw and enlarged submandibular lymph node and one surviving female rat which showed nose bleeding, corneal opacity of a bloody left eye, and a hairless region around the eye.

Systemic arteritis was observed at 12, 30, and 80 mg/kg bw/d, each in one female rat that died on day 24, 38 and 25, respectively, and was not considered to be treatment-related. Slightly reduced food consumption and 9-10% lower body weight gain in comparison to control values were observed in male rats at 150 mg/kg bw/d, while no treatment-related effect on the body weight was seen on any of the female dose groups. These findings do not indicate nonspecific toxic effects.

This study is difficult to interpret as the day of death is not given for all decedents and as the toxicity/mortalities occurred without a clear dose response. As far as the data are reported, the lesions in the laryngo-pharyngeal regions were seen in animals that died on day 1, 52 and 75 of treatment. Either all the effects from 30 mg/kg bw/d onwards were considered substance related or interpreted as being related to the pre-gastric (mal-)administration (at least of parts of the applied dose) of the high concentration of RP 1:1, in the absence of a clear dose-relationship of the observed clinical and histopathological effects and considering the small incidences and the pharyngeal/oesophageal sites (lesions due to assumed irritative properties following a gavage administration would be expected to occur in the forestomach) affected in animals that died.

The test substance concentration at 150 mg/kg bw/d was 6% in peanut oil.

It is the opinion of RAC that for RP 1:1 no clear conclusion on oral repeated dose toxicity can be drawn from this study.

The pharynx/larynx was also examined in the second 90-day study (Schülke & Mayr, 2001). The DS interpreted this study as not valid as the Maximum Tolerable Dose (MTD) was not clearly reached and no local GI tract effects were seen. The absence of local effects in the upper GI tract after gavage administration with doses up to 180 mg/kg bw/d at concentrations up to 2.5% in water as the vehicle, may be related to the less concentrated test material and/or to the lack of maladministration.

No treatment-related mortality was observed at 0, 40, 100 or 250 mg/kg bw/d. High dose females showed a decreased motor activity (measured). Food consumption was

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significantly lower in males of the mid and high dose group at week 11, a slight dose-dependent decrease in bw gain was seen during the last 3 weeks of the treatment period for the high dose males (-9%) and mid and high dose females (-8%). Several effects on haematology, clinical chemistry and organ weights were reported. However, the study seems to be of limited value due to varying degree of pneumonic changes with histopathological characteristic of mycoplasma pneumoniae that was indicated in the study report according to a note of the Rapporteur Member State (RMS).

No conclusion with regards to the classification for STOT RE can be drawn from two range-finding 14-day studies (Becker Chemie, 2002; Schülke & Mayr, 2002).

Formaldehyde

There is no harmonised classification on formaldehyde for STOT RE.

Lesions related to the irritancy in the stomach are - similar to RP 3:2 - the main effects after repeated oral administration of formaldehyde. However, available studies suggest that the lesions were seen at comparatively higher doses or occurred with lower severity grades compared to RP 3:2.

After 12 months exposure to 300 mg/kg bw/d, forestomach squamous cell hyperplasia/hyperkeratosis, glandular hyperplasia and erosion/ulceration of the glandular stomach were seen (Tobe *et al.*, 1989, Doc III A6.3.1). No local effects in the GI tract were observed in a 90-day study in rats receiving drinking water with formaldehyde up to concentrations of 1000 mg/L (150 mg/kg bw/d) (Johannsen *et al.*, 1986). A 4-week oral study in rats (Til *et al.*, 1988, formaldehyde Core Doc III A6.3.1) receiving 0, 5, 25, 125 mg/kg bw/d with drinking water revealed at 125 mg/kg bw/d very slight to moderate hyperkeratosis of the forestomach (all animals) and very slight to moderate gastritis (3/10 males, 5/10 females) of the glandular stomach. A focal papillomatous hyperplasia was observed in one female. None of the available studies conducted were fully compliant with the relevant guidelines.

2-Hydroxypropylamine

The NOAEL of 600 mg/kg bw/d was estimated in a 90-day feeding study with limitations, being conducted in rats long before the OECD standards on testing were developed (Smyth *et al.*, 1951). Alterations (without further details) in kidney and liver were observed at 2200 mg/kg bw/d.

RP 3:2

The most relevant study is a 90-day study (Bode Chemie, 2002, in accordance to OECD TG 408, version 1998) on 10 male and 10 female rats which resulted in mortalities of 3 males (day 49-75) and 5 females (day 49-79) that received 180 mg/kg bw/d (high dose, reduced to 120 mg/kg at week 12). No mortalities were seen in the low and mid dose groups (mid dose 60 mg/kg bw/d). These doses corresponded to concentrations of 0.4%, 1.2% and 3.6/2.4% in corn oil for the low, mid and high dose groups. It is to be noted that effects at these concentrations would not lead to classification as skin irritant as the concentration is below 5%.

The study has weaknesses as the stomach and the bone marrow were the only organs examined for histopathological effects in the low and mid doses. Histopathology findings were reported (Doc III A6.4.1/02) without any grading of severity and with lack of information, such as whether all animals with ulcerative gastritis had also peritonitis.

All males and females of the high dose groups showed long-lasting piloerection from day 35 onwards. Ataxia was noted in one female. Reduced pupil size was detected in 3/7 male and 5/5 female survivors. Clinical abnormalities from the functional observational battery give some indications on abnormal neuromotor and sensory functions at 180/120 mg/kg bw/d. Gait impairment in one female and reduced pupil size (miosis - loss of

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capacity to adapt to darkness due to permanently contracted pupils) were seen in 3/7 males and 5/5 female survivors. The study authors interpreted these effects as being of unclear toxicological relevance that occurred at doses greater than the MTD.

The view of RAC is that a neurotoxic effect could not totally be excluded, as the effects were seen in surviving animals (after week 11) and miosis is not considered to be associated with gastritis. As the dose of 180 mg/kg bw/d during the first 11 weeks is above the guidance value limit for classification as STOT RE (100 mg/kg bw/d for a 90-day study), these effects do however not warrant classification.

In principle the mortalities at 180/120 mg/kg bw/d that occurred at day 49 or later could be relevant for classification for STOT RE, as they could not be seen as acute toxic effects. As the toxic effects at the high dose (including the ulcerative gastritis, peritonitis and a shift to higher relative numbers of neutrophilic granulocytes and reactive bone marrow granulopoiesis) occurred at above the upper limit of the guidance values (100 mg/kg bw/d for a 90-day study), they do not justify classification. Granulocytosis and increased granulopoiesis are likely to be secondary systemic effects to the chronic inflammatory and ulcerative processes in the stomach and peritonitis.

Local effects in the stomach were also observed in about half the animals (6 males, 5 females) treated with 60 mg/kg bw/d (1.2% in corn oil). In addition, increased medullary granulopoiesis was seen in 4 males and one female at this dose. Repeated exposure to low concentrations that are not irritant at single exposure conditions may lead to exacerbations of adverse effects which over time may result in toxicologically significant effects. These chronic lesions could be relevant for classification for STOT RE. The DS argued that 60 mg/kg bw/d is more than half an order of magnitude lower than the dose leading to the acute toxicity and that the local effects are sufficiently addressed by classification for corrosion/irritation.

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

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

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

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

The dose at which the effects in the stomach and bone marrow occurred in the 90-day study was much lower than the oral acute toxic doses (LD₅₀ 630 mg/kg bw). Local effects in the stomach were not observed in three oral acute toxicity studies (at much higher test concentrations of 8% - 10%, highest dose tested was 2000 mg/kg bw). RAC, in line with comments received during the public consultation from some MSCAs, does not agree with the DS' view that the local irritant effects are mechanistically sufficiently addressed with the classification for corrosion and therefore should not support classification for STOT RE.

The toxic effects in the GI tract are considered as chronic toxic effects that resulted from prolonged/repeated exposure to low concentrations/doses of RP 3:2. The effects are considered to reflect repeated exposure toxicity and not just acute toxicity. Because they

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occurred within the range of guidance values (CLP regulation, Table 3.9.2-a, ≤ 100 mg/kg bw/d for an oral 90-day study) and the effective dose is considerably lower than the acutely toxic dose, RP 3:2 should be classified for STOT RE. Local effects in the GI tract (like chronic oesophagitis, gastritis) after repeated/prolonged exposure are toxicologically relevant as they impair not only the morphology and/or function of the locally targeted organ, but also bear the potential to impair adherent tissues/organs by transmural extension of the chronic inflammation (e.g. peritonitis, pleuritis) or to cause delayed mortalities (after ulceration into body cavities). Thus, RAC agrees with the proposal from an MSCA during public consultation to classify RP 3:2 as **STOT RE 2; H373 (May cause damage to the GI tract through prolonged or repeated exposure)**.

Dermal route

RP 1:1

No repeated dose study using the dermal route is available.

Formaldehyde

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

2-Hydroxypropylamine

No repeated dose study using the dermal route is available.

RP 3:2

No repeated dose study using the dermal route is available.

Taking the data from formaldehyde into account, the overall database is not sufficient to take any decision on classification for STOT RE for this route.

Inhalation route

RP 1:1

No repeated dose study using the inhalation route is available.

Formaldehyde

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

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

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

In addition, section 3.9.2.5.1 gives guidance on the relevant doses:

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

In short, if doses are considerably lower than those causing acute toxicity/irritation and these low doses induce serious health damage after repeated inhalation with accumulation/ exacerbation of repeated exposure, classification for STOT RE should be considered.

For formaldehyde, the acute inhalation LC₅₀ was reported to be 0.6 mg/L (600 mg/m³) by Nagorny *et al.* (1979) (see Formaldehyde Core Document II, Table 3-2). Taking the adverse effect concentration (AEC) of 0.12 mg/m³ from human data into account, the surrogate effect for repeated inhalation toxicity occurs at concentrations 5000-fold below the acute toxic dose, thus indicating that a classification for repeated inhalation effects is warranted.

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

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

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

In repeated dose inhalation studies in animals, dose-dependent non-neoplastic lesions in the nasal cavity that increased in severity and extension with exposure time and dose (for review see SCCS, 2014; BfR, 2006) were reported. Following inhalation exposure up to 24 months, squamous metaplasia was observed in rats at 6 ppm formaldehyde. Epithelial hypertrophy, hyperplasia and metaplasia, mixed inflammatory cell infiltrates, and turbinate adhesions were seen at 10 ppm; in addition destructed turbinate architecture occurred at 15 ppm (Monticello *et al.*, 1996, cited from BfR, 2006). While lesions of the respiratory epithelium in the nasal cavity were not reported after 6 weeks exposure up to 2 ppm (Monticello *et al.*, 1991; Formaldehyde Core document IIIA), inhalation exposure of ≥ 12 months to ≥ 2 ppm (2.456 mg/m³) formaldehyde caused purulent rhinitis, epithelial dysplasia and squamous metaplasia at level I of the nasal cavity (Kerns *et al.*, 1983a, b, cited from BfR, 2006). At higher concentrations than 2 ppm, lesions extended to more posterior parts (level I to III) of the nose and reached the trachea at 14.3 ppm. Monticello (1989, cited from RAC Opinion on formaldehyde) has demonstrated that inhalation of 6 ppm formaldehyde for 1 or 6 weeks induced loss of cilia, inflammatory response, epithelial hyperplasia and squamous metaplasia and increased cell proliferation in the nasal passages of rhesus monkeys. Like in rats, lesions in monkeys showed an anterior-posterior gradient and a duration-related increase in

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severity and extension, but these were more widespread than in rats. Inhalation of 3 ppm formaldehyde over 26 weeks induced squamous metaplasia and hyperplasia in the nasoturbinate in 6/6 Rhesus monkeys, while no effects were observed at 0.2 and 1 ppm (Rusch *et al.*, 1983, see SCCS, 2014).

Taking 2 ppm formaldehyde as a robust LOAEC for chronic inflammatory and meta/hyperplastic lesions secondary to initial cytotoxicity in the nasal mucosa from repeated/prolonged inhalation, and using the Haber's rule standard extrapolation from 12 months to 90 days exposure when comparing with the guidance values, 2 ppm for 12 months corresponds to 8 ppm ($9.824 \text{ mg/m}^3 = 0.01 \text{ mg/L}$) for a 90-day study. This is clearly below the guidance concentration for gases of 50 ppm and would justify a classification of formaldehyde as STOT RE 1.

2-Hydroxypropylamine

There are no repeated dose inhalation studies with test guideline conformity.

Bronchopneumonia and rhinitis were observed in two 11-day inhalation studies in rats and mice. The same effects seen in the control groups invalidate these studies (Doc Appendix HPA).

RP 3:2

No repeated dose study using the inhalation route is available.

The DS suggested read across to the hydrolysis product formaldehyde on which a local inhalative AEC of 0.12 mg/mg^3 was based on human data on eye irritation.

Referring to the CLP Regulation, 3.9.2.10.3, RAC agrees with the DS on the read across to formaldehyde as data on repeated dose inhalation toxicity of RP 3:2 are lacking. However, RAC does not agree that effects after repeated inhalation exposure are covered by the classification for corrosion.

The absence of an entry for formaldehyde for STOT RE in CLP, Annex VI does not by itself justify non-classification for RP 3:2.

The DS informed that RP 3:2 contains about 45% releasable formaldehyde. Assuming that under prolonged inhalation exposure conditions RP 3:2 would continuously release the maximal releasable amount of 45%, a factor of 2.2 should be applied to correct for the lower content of formaldehyde. As the human AEC was based on eye irritation, an acute receptor-mediated sensory irritation effect (without obvious cytotoxicity and infiltration of inflammatory cells) as surrogate for the lowest adverse effect in humans, animal data on repeated inhalation toxicity may be more appropriate to conclude on the classification for STOT RE.

For RP 3:2, the LOAEC for repeated inhalation exposure is based on the LOAEC of 2 ppm for formaldehyde (2.456 mg/m^3 , derived from a rat 12-month study; Kerns *et al.*, 1983 a,b) which corresponds to 8 ppm ($9.824 \text{ mg/m}^3 = 0.01 \text{ mg/L}$) for a 90-day inhalation based on Haber's rule. The 8 ppm LOAEC corrected for the maximal amount of releasable formaldehyde from RP 3:2 (45%) with a factor of 2.2 results in a (corrected) concentration of 0.02 mg/L for RP 3:2 which is at the lower boundary of the guidance value (for mists) for STOT RE 2 ($0.02 < C \leq 0.2 \text{ mg/L}$). As inhalation exposure to the aerosol is expected to be the main concern for RP 3:2, the guidance values for the gaseous form were not considered.

If the chronic toxicity occurred at the same dose level as the acute inhalation toxicity, chronic toxicity would be covered by the classification for acute toxicity. The inhalative LC_{50} was unknown for RP 3:2 (and RP 1:1) as no acute inhalation study is available. As a

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substitute information on the difference between the level of the inhalation LC₅₀ and the LOAEC for chronic effects for formaldehyde is considered. The Formaldehyde Core Document indicates a LC₅₀ of 0.6 mg/L (4 h) which is markedly higher than LOAEC for chronic effects (2 ppm = 2.456 mg/m³). Thus the acute toxicity classification does not cover the classification for STOT RE.

It is noted that the formation of formaldehyde as hydrolysis product may depend on several factors (e.g. temperature, pH, dilution). The RMS raised some uncertainties related to the data (Doc II A 2.12), namely that exposure conditions or hydrophobic formulations may reduce the rate of hydrolysis, but may theoretically enhance deeper respiratory tract exposure and may also increase irritation properties due to their effect on membranes.

Repeated inhalation to RP 3:2 generates the hydrolysis products formaldehyde and 2-hydroxypropylamine. Whether 2-hydroxypropylamine may exert additive effects to those expected from formaldehyde, remains unknown.

Based on the read across from data on formaldehyde, RAC agrees to classify RP 3:2 for the effects on the respiratory system as STOT RE 2; H373

All routes/Overall classification on STOT RE

If classification for STOT RE is proposed based on data from several routes with different target organs, the final labelling should consider all the relevant target organs. RAC agrees that classification of RP 3:2 is warranted as **STOT RE 2; H373 (May cause damage to the respiratory tract and the gastrointestinal tract through prolonged or repeated exposure)**.

No specific route should be indicated.

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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).

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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).

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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	Frequen- cy of applica- tion	sampling times	dose levels	identity as given in study report	Results dose, sampling time and result +/- /±	Remarks	Reference
Mouse bone marrow mic- ronucleus test; OECD 474	Mouse NMRI 5 m & 5 f	Single i.p. applica- tion	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

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bone marrow chromosome aberration test; OECD 475	NMRI 5 m & 5 f	application	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 applications (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.

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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.

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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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter’s proposal

The DS proposed to classify RP 3:2 as a category 2 mutagen based on the existing harmonised classification of its hydrolysis product formaldehyde.

There are several mutagenicity studies *in vitro* and *in vivo* for RP 3:2. Predominantly, clastogenic effects were induced in cells of mammalian cell cultures whereas bacterial gene mutations tests were weakly positive (one test) or negative (two tests). Regarding the *in vivo* testing, a negative *in vivo* micronucleus test and an *in vivo* chromosomal aberration test, which was assessed as equivocal by the DS, are available. The *in vivo* data provide no clear evidence for the induction of mutagenic effects since there is limited information that the active substance reached the bone marrow cells in relevant concentrations.

The DS additionally provided information on similar results of *in vitro/in vivo* mutagenicity tests for the substance RP 1:1. (To avoid a confusion it should be noted

that the corresponding references in the tables 4.8-1 and 4.8-4 were taken from the CLH report for RP 1:1. They are not part of the reference list of the CLH report for RP 3:2).

The DS also argued that due to the rapid hydrolysis of RP 3:2 to formaldehyde at contact with biological tissues, an induction of local genotoxic effects is to be expected at the site of first contact *in vivo*. Therefore the DS refers to the classification of formaldehyde that is classified as a category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at the site of contact, and supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. The other hydrolysis product 2-hydroxypropylamine is of very minor toxicological relevance.

Due to the mechanistic considerations of formaldehyde release from RP 3:2 the DS proposed to classify RP 3:2 as a Muta. 2 on the basis of its hydrolysis product formaldehyde.

Comments received during public consultation

Two MSCAs expressed their support for the proposed classification. One individual commenter disagreed with the proposed classification as Muta. 2 due to the lack of relevant mutagenicity data.

Assessment and comparison with the classification criteria

RP 1:1

RAC takes note of the additional information by the DS that RP 1:1 induces similar results in mutagenicity tests *in vitro* and *in vivo* as RP 3:2.

Formaldehyde

RAC agrees with the approach of the DS to take into account the classification of formaldehyde as a category 2 mutagen in the justification of the classification of RP 3:2.

Formaldehyde that is rapidly released from RP 3:2 on contact with biological tissues is classified as a category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at the site of contact, supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. Although it seems likely that the amount of formaldehyde may vary depending on the use, the inherent potential of RP 3:2 to release formaldehyde is a critical factor.

Testing of the *in vitro* mutagenicity of RP 3:2 shows that the observed positive effects are consistent with those known from formaldehyde alone. Uncertainties remain due to the relevance of the available (negative) *in vivo* studies. However, it is assumed that RP 3:2 has poor systemic availability *in vivo* due to its rapid hydrolysis. Therefore it seems unlikely that genotoxic effects are induced at a site distant from first contact.

Although no distinct rule is noted on reaction products from UVCBs in the CLP regulation, (likewise for CMR substances in mixtures, Art 6.3 of the CLP Regulation and Section 1.6.3.1 of the CLP Guidance) the information on the hydrolysis product was used to assess the mutagenic potential of RP 3:2.

2-hydroxypropylamine

The DS noted that no indication for mutagenicity of 2-hydroxypropylamine has been detected in available bacterial studies and no relevant structural alerts are present.

RP 3:2

The evaluation of the mutagenicity data of RP 3:2 by the DS and RAC does not differ. RAC also comes to the conclusion that a proposal for classification of RP 3:2 as a

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category 2 mutagen is justified.

In vitro data

The available bacterial gene mutation tests are weakly positive with and without S9-mix (Lubrizol Corporation, 2000, see Doc III A6.6.1/03) or negative (Schülke and Mayr, 1997, Doc IIIA 6.6.1/01; Schülke and Mayr, 2000, Doc III A6.6.1/02). It is not possible to conclude on the relevance of the negative results because the tested concentrations were below the highest concentration (5000 µg/plate or relevant cytotoxic concentration) recommended by the respective test guideline.

Two mouse Lymphoma assays (Lubrizol Corporation, 2001, Doc III A6.6.3/02; Schülke and Mayr, 2002, Doc III A6.6.3/01) were positive with and without S9-mix. At the analysis of the colony sizes, predominantly small colonies were found, which indicate clastogenic activity of RP 3:2.

A chromosomal aberration test was positive in CHL cells with and without S9-mix (Lubrizol Corporation, 2001, Doc III A6.6.2).

In vivo data

Two studies are available that are able to detect systemic chromosome mutagenic activity in bone marrow cells of mice:

- One *in vivo* micronucleus was negative after single gavage up to the highest tested dose of 300 mg/kg bw, which induced clinical symptoms (Bode Cemie, 2002 (DocIIIA6.6.4/02)). Due to the lack of cytotoxic effects (reduction of PCE/NCE ratio) and due to the lack of detailed information on the nature and severity of clinical symptoms, no conclusion can be drawn as to whether the MTD was reached.
- One *in vivo* chromosomal aberration test after repeated gavage of RP 3:2 at the highest tested dose of 367 mg/kg bw, in which an increase of the chromosome aberration frequency was observed (not statistically significant; Schülke and Mayr (Doc III A6.6.4/01), 2002). Neither cytotoxic effects nor clinical symptoms were induced. Thus the highest tested doses did not correspond to the MTD nor was it in accordance with highest guideline recommended dose.

The quantity of test data for RP 3:2 is limited and the mutagenicity studies are not published. Thus, only the data given by the applicant in the biocide registration dossier are available. These data allow neither a detailed test evaluation nor they do allow to assess whether a test performance is fully in accordance with the corresponding test guideline. But despite these limitations, the following conclusion can be drawn: in bacteria as well as in somatic cell cultures, mutagenic effects are induced. The results from an *in vivo* micronucleus test as well as from an *in vivo* chromosomal aberration test are of limited relevance due to obvious methodological deficiencies.

RAC discussed that due to its reactivity a poor systemic availability is expected for RP 3:2 and therefore, the induction of systemic genotoxic effects is unlikely. However, a local genotoxic effect produced by the hydrolysis product formaldehyde is expected and RAC considers read across to formaldehyde, which is classified as Muta. 2 based on its local genotoxic action, justified. Some RAC members expressed that the CLP guidance text relates only to classification of substances that caused germ cell mutations. This view is reflected in a minority position supported by two RAC members. RAC recognised that according to the guidance, section 3.5.1, classification is also warranted if there is evidence of only somatic cell genotoxicity leading to classification in category 2 if genotoxic substances are only acting locally.

RAC agrees with the proposal of the DS to classify RP 3:2 for **Germ cell mutagenicity, category 2; H341 (Suspected of causing genetic defects)** based on the properties of its hydrolysis product formaldehyde.

Supplemental information - In depth analyses by RAC

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

RP 3:2 has poor systemic availability due to its quick hydrolysis. Accordingly, the available *in vivo* results are of low relevance and do not allow the conclusion that the substance is not genotoxic in the whole animal. There is no test with RP 3:2 that examines the possible induction of genotoxic effects in cells at the site of first contact. But for the toxicological evaluation of RP 3:2 it is taken into account that its hydrolysis product formaldehyde is already classified as a category 2 mutagen due to the induction of local genotoxic effects.

4.9 Carcinogenicity

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

No long-term carcinogenicity 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/m ³
Systemic carcinogenicity in humans	No data	No data	Conflicting results
Local carcinogenicity in	No data	No data	Conclusion from not

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humans			unequivocal epidemiological studies: increased tumour risk after inhalation exposure
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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	Formaldehyde -releasers are designed as transport

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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 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%

In vitro genotoxicity data for MBM support the assumption of local genotoxicity and consequent local carcinogenicity

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 in dilutions with water

→ depending on the releaser type this needs dilutions between 1:10 and 1:1000

Other examples for substances (oligomers) that contain formaldehyde and are classified according to free formaldehyde:

- 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

Genotoxicity 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

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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.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter’s proposal

No cancer bioassay or human data were available for either RP 1:1 or RP 3:2. The DS discussed arguments that the classification should relate to the substance itself (consider only free formaldehyde) and not to potentially released or degraded substances (proposal 2, p. 48 of the CLH report). Also, arguments supporting a classification based on the hydrolysis to formaldehyde are reflected and in the end taken forward. The proposal was to classify in line with formaldehyde

Comments received during public consultation

Three MSCA agreed and four industry commenters disagreed with the classification proposal.

Assessment and comparison with the classification criteria

RP 1:1

No carcinogenicity studies are available on RP 1:1.

Formaldehyde

The hydrolysis product formaldehyde is classified in CLP, Annex VI for Carcinogenicity, category 1B.

2-Hydroxypropylamine

No information on the carcinogenic potential of 2-hydroxypropylamine is available.

RP 3:2

There are no reliable human data. Two medical letter reports in Doc IIIA stated that no adverse effects have been documented from annual medical screenings of employees in the manufacturing of products containing formaldehyde releasing biocides or with the active substance RP 3:2. No information is given on the details of the level, duration, frequency and conditions of exposure, the substances the workers were exposed to or the details of the medical examinations and results.

No studies on carcinogenicity or prolonged/repeated inhalation exposures are available for RP 3:2. The non-submission of data was justified by a read across to formaldehyde and probable carcinogenic effects of RP 3:2 are considered by the applicant in the biocide process to be related to the hydrolysis product formaldehyde (Doc III A6.7).

It is expected that RP 3:2 exerts similar effects as formaldehyde such as cytotoxicity, hyperplasia, metaplasia, tumours and local mutagenic effects at the sites of contact - on the epithelium of the respiratory tract following prolonged inhalation as formaldehyde is one of the main hydrolysis products.

It is assumed for RP 3:2 that, similar to formaldehyde, systemically increased bioavailability and a concern for systemic carcinogenic responses are not to be expected.

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Although it is noted that the amount of formaldehyde may vary depending on different uses, the reaction product of paraformaldehyde and 2-hydroxypropylamine is intended to release formaldehyde in aqueous solutions. In aqueous solutions RP 3:2 is expected to hydrolyse completely under environmental conditions or when the substance has entered the human or animal bodies (Doc IIA1.4.2). Both hydrolysis products, formaldehyde and 2-hydroxypropylamine, are considered to be slightly volatile from aqueous solutions.

The following is presented as clarification of the objectives of the classification proposal and in response to some comments received during public consultation. Exposure to formaldehyde may result from inhalation or dermal exposure to RP 3:2 as an active substance. This can result from exposure to the undiluted UVCB substance, and (as considered in the CLH report) the contact with biological tissues/media then generates hydrolysis products (including formaldehyde). Similarly, exposure to RP 3:2 in aqueous solution (such as diluted formulations or products on the market) can result in contact with hydrolysis products from the dilution and with those directly generated following contact with biological media. Exposure to the gaseous form after evaporation of formaldehyde from the undiluted or diluted RP 3:2 can coincide with the above.

Formaldehyde is classified based on its carcinogenic potential at the sites of exposure, primarily on the nasopharyngeal tumours observed in man and rodents after prolonged inhalation⁴.

The CLP guidance, Section 3.6.2.2.7 states:

"A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes."

The CLP guidance (section 1.4.3) explicitly foresees the read across of information from 'source' substances to predict the same hazard for another 'target' substance. For RP 3:2, it is not about the similarity of source and target substance, but RP 3:2 should be classified as a carcinogen based on the release of the *identical* substance (formaldehyde) resulting from hydrolytic transformation of RP 3:2.

Endpoints, on which data on RP 3:2 are available, show that effects were consistent with those known from formaldehyde alone. Similar effects were noted e.g. for the oral repeated dose toxicity, with the observation that the toxicity may be more severe for RP 3:2 when comparing with the dose levels or the severity of effects observed with formaldehyde. However uncertainties remain due to the lack of studies with full guideline compliance and as an additional contribution of the other hydrolysis product 2-hydroxypropylamine to the effects by formaldehyde are unknown.

As mentioned by the DS, from a quantitative aspect, the hydrolysis rate of RP 3:2 to formaldehyde depends on several environmental factors (increase at higher temperature, lower pH, and at higher dilution). At all tested pH levels the hydrolysis half-life was less than 1 h. However, water contact or dilution of RP 3:2 with aqueous solutions are not a necessary condition for exerting toxic effects of RP 3:2, for the aerosol aqueous conditions were given at contact sites (mucous membranes with oral & inhalation

⁴ http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling?search_criteria_name=Formaldehyde&search_criteria_ecnumber=200-001-8&search_criteria=Formaldehyde

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exposure, sweaty skin with dermal exposure) and as demonstrated by similar toxic effects with lipid vehicles. The CLH report stated that the equilibrium of RP 3:2 (or RP 1:1) shifts towards formaldehyde with dilution and with the reaction of formaldehyde with biological media.

To the industry representative comment during public consultation that a classification of RP 3:2 as a carcinogen is not justified based on the end use 'diluted metal working fluid' containing less than 0.1% of 'free, unbound' formaldehyde and the slow rate of formaldehyde release during its use, the DS replied that the end uses and different forms of the substance that are on the market are not relevant for the decision on classification.

The industry representative further stated that evidence is lacking that sufficient formaldehyde will be released during exposure to workers to cause a carcinogenic risk. RAC notes that the CLP Regulation states that classification is based on intrinsic hazards of a substance and does not take the exposure conditions into account.

The option to classify RP 3:2 as carcinogen, in category 2, in order to account for uncertainties for substances that are unstable, showing equilibrium behaviour and having half-lives depending on dilution, temperature and pH (as was discussed in the CLH report) is not supported by RAC. Taking into account the evidence from read across to the specific substance (and hydrolysis product) that is known to have carcinogenic properties (formaldehyde), no reasons (such as uncertainty about structural similarity or qualitative differences in the mechanistic aspects) could be identified to justify a downgrading of the classification into category 2.

Hydrolysis tests demonstrated that high concentrations of formaldehyde are generated within short time periods. These hydrolysis tests support qualitatively that hydrolysis will occur in contact with aqueous biological media on mucous membranes. Inhalation exposure to aerosolic RP 3:2 is expected to result in hydrolysis at the site of contact and toxicologically significant concentrations of formaldehyde could be reached on surface of the mucous membranes in the respiratory tract, eye or upper GI tract or skin. The inhalation exposure to gaseous formaldehyde that evaporated from RP 3:2 is assumed to contribute in addition to the toxic/carcinogenic effect resulting from the direct impact of hydrolysis products at the contact site. Demonstrating that the room concentrations of released gaseous formaldehyde are rather low would not be sufficient to discount the hazardous potential that may result from the inhalation exposure to RP 3:2 aerosol.

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

Although no specific mention is made on classification of reaction products from UVCBs in the CLP Regulation, (likewise for CMR substances in mixtures, Art. 6.3 of the CLP Regulation and section 1.6.3.1 of the CLP guidance) information on the hydrolysis product is used here to assess the hazardous properties including the carcinogenic potential of RP 3:2. More guidance is given in REACH, Annex XI, 1.5.2 that specifies that similarities to substantiate the read across may be based on common precursors or common breakdown products via physical or biological processes, which results in structurally similar chemicals.

RAC agrees with the proposal of the DS to classify RP 3:2 as **Carcinogen, category 1B; H350 (May cause cancer)**.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information – 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.

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4.10.1.2 Non-human information – RP 3:2

Table 4.10-1 Summary of data for potential fertility effects

Route of exposure	Test type 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. Doc IIIA6.4.1/02; 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 resorptions 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.

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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 $\sim 0.1\%$ 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 Himalayan female 24	Gestation day 6-28	0, 5, 45, 90, 135 mg/kg bw	GrotaMar 71 Batch 1094394 Purity 99%	Local effects in the stomach No teratogenicity	5 mg/kg bw/day	90 mg/kg bw/day	Lubrizol Deutschland GmbH (2006); DocIII A6.8.1

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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/m ³) NOAEL 20 ppm (24 mg/m ³) developmental effects LOAEL 39 ppm (47 mg/m ³) NOAEL 20 ppm (24 mg/m ³)
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-hydroxypropylamine (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.

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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 gastrointestinal 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.

4.10.4 Comparison with criteria

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

4.10.5 Conclusions on classification and labelling

No classification for reproductive toxicity is necessary.

RAC evaluation of reproductive toxicity
<p>Summary of the Dossier submitter's proposal</p> <p>One developmental toxicity (OECD 414) and one fertility study (OECD 415) were available which had been conducted using RP 3:2. According to the DS, these studies did not support classification for either sexual function and fertility or development. No reprotoxicity study are available for 1:1.</p> <p>Comments received during public consultation</p> <p>One MSCA commented that the DS's interpretation of implantation loss and post-natal deaths at the high dose as being secondary to the maternal effects was not substantiated and suggested conducting a comparison of individual dam and fetal/pup effects.</p> <p>RP 3:2</p> <p><u>Effects on fertility</u></p> <p>The documentation of the results of the 1-generation study in the CLH report was not sufficiently detailed to enable a conclusion to be reached on whether the fertility effects are direct or secondary effects or whether or not classification is justified.</p> <p>To strengthen the database, more details from the MBO Doc III A and from the original study report are given in the BD.</p>

Additional key elements

A 1-generation study conducted according to OECD TG 415 RP 3:2 (in corn oil) was administered to rats by gavage at 0, 5, 15, 45 mg/kg bw/d for 70 days before pairing, during pairing (max. 14 days), a 37-day period after pairing (for males) and until weaning on day 21 (females).

The nominal concentrations of the applied RP 3:2 doses were 0.1%, 0.3% and 0.9% (see MBO Doc III A 6.8.2_1). No reliable information on the actual concentrations is available as they were estimated with a more than 1 year after the end of the study. The estimated actual concentrations (0.5%, 2% and 7%) appear to be rather high; degradation was assumed by the reporting Member State (RMS) and the DS.

No increased mortalities or clinical signs were reported. Forestomach lesions were observed in male rats at 15 mg/kg bw/d and in female rats at 45 mg/kg bw/d.

In males, mean food consumption was slightly and occasionally significantly reduced during the prepairing period and body weight gain was slightly (non-significantly) lower than control values (day 70: 208 g vs. 223 g in controls). No effect on the body weight was seen after pairing (until day 37). Minimal to slight vacuolar degeneration and minimal squamous hyperplasia, hyperkeratosis, submucosal inflammation and oedema was recorded in up to 5 of 24 males at 15 mg/kg bw/d. The study summary stated that one male of this dose group showed minimal focal erosion, however, none was documented in the summary table. Males at 45 mg/kg bw/d had slightly reduced food consumption in the pre-pairing phase (-5.8%) and slightly reduced bw gain, and 19/24 males showed moderate to marked ulcerations, minimal to slight vacuolar degeneration and squamous hyperplasia with hyperkeratosis, submucosal inflammation and oedema of the forestomach. Corresponding macroscopic lesions were seen in the stomach of 17 males.

In female rats, no effects were reported at 15 mg/kg bw/d. One high dose dam (No. 185) showed lethargy, diarrhea and dystocia. Female rats at this dose (45 mg/kg bw/d) showed slightly (non-significantly) reduced food consumption during the last gestational week (-6.3%). Mean body weight (and bw gain) was not affected. Stomach lesions were macroscopically noted in 2 out of 24 females of the high dose group. Microscopically minimal to moderate squamous hyperplasia (5 rats) with hyperkeratosis (7 rats), submucosal inflammation (2 rats) and oedema (1 rat) were observed in females of this dose group. The evaluation of the RMS (in MBO Doc IIIA 6.8.2_1) in agreement with the individual animal data of the study report outlines that 4/24 females showed ulcerations of the forestomach.

In their evaluation, the RMS (MBO Doc IIIA 6.8.2_1) also noted that '*additionally females were observed for signs of difficult or prolonged parturition, and behavioural abnormalities in nesting and nursing*' without giving information on the dose groups affected. No information related to the clinical abnormalities around parturition could be found in the summary of the study in MBO Doc IIIA 6.8.2_1 and the study report (except the one case with dystocia (No. 185)).

No effect was seen on the fertility index (no. of pregnant females/no. of females cohabited) (87.5% in controls, 91.7%, 87.5% and 91.7% in the low, mid and high dose groups, respectively).

Post-implantation losses increased dose-dependently in all dose groups (7.5% in controls, 12.3%, 14.7%, and 16.3 % in the low, mid and high dose groups, respectively). The total number of lost implantations were 20, 35, 41 and 47, respectively, with significant increases in all dose groups. No treatment-related effect was seen on the number of litters affected (10, 12, 14 and 10 in the control, low, mid and high dose groups, respectively).

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At the first litter check (Day 0/1) the pup viability was decreased in the mid and high dose groups. The total number of dead pups were 3 in controls and 2, 12, and 24 in low, mid and high dose groups and mean number of dead pups were 0.1, 0.1, 0.6 and 1.1, respectively. The number of litters affected were 3, 1, 5 and 6 in the control, low, mid and high dose groups, respectively.

No treatment-related effect on pup survival was seen at Day 4. The high post-natal loss until Day 4 in the control group (27 dead pups, 10.9%) was noted by the RMS in the MBO Doc IIIA. The original study report indicated that the 26/27 mortalities in the control pups stem from 3 litters (No. 109 10/13 deaths on Day 2; No. 112 12/13 deaths on Day 2 (thereof 9 missing), 1/13 death on Day 1; No. 120 3/11 missing on Day 2, 1 death on Day 1). Additional information indicated that the mean of historical controls in 17 control groups was 98.9% (it was assumed that this information came from the Harlan laboratories; no information on the years when these were conducted available). This study was found to be within the historical control range, as one out of the 17 studies revealed also a viability index of 89.1%.

In addition, no clear-dose relationship was found on the pup survival of treated groups from Day 1 to Day 4 (if considered separately from survival at Day 0/1) when comparing the no. of dead pups in the low, mid and high dose groups (4%, 2.9% and 7.9%, respectively).

Table: Summary on maternal toxicity (corresponds to the upper part of Table A6_8_2-2 MBO Doc IIIA 6.8.2_1)

Parameter		Generation	control		low dose		medium dose		high dose	
			m	f	m	f	m	f	m	f
Mortality	incidence	P	0	0	0	0	0	0	0	0
Food consumption (preparing period, days 1-70)	% of control	P			-1.5	+1.5	-1	+2.9	-5.8	+1.5
Food consumption (females, last gestational week, day 14-21)	% of control	P				+5.2		+2.9		-6.3
Differences in mean body weight gain (preparing period, from day 1 to 70)	% of control	P	107	+55	+103	+54	+107	+56	+101	+57
Clinical Observations	Incidence	P	n.s.f.	n.s.f.	n.s.f.	n.s.f.	n.s.f.	n.s.f.	n.s.f.	1#
Pathology		P								
Macroscopic findings (Stomach)		P								
Cratiform retractions	Incidence, (%)	P	0	0	0	0	0	0	9(38)	1(4)
Isolated cratiform retractions	Incidence, (%)	P	0	0	0	0	0	0	3(13)	1(4)
Nodules	Incidence, (%)	P	0	0	0	0	0	0	5(21)	0
Several cratiform retractions	Incidence, (%)	P	0	0	0	0	0	0	1(4)	0
Histopathologic examination (Forestomach)										
No. animals examined		P	24	24	24	24	24	24	24	24
Ulcerations	Incidence*	P	0	0	0	0	0	0	19 (4.1)	4 (3.3)
Vacuolar	Incidence	P	0	0	0	0	4	0	5	0

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degeneration							(1.8)		(1.6)	
Squamous hyperplasia	Incidence	P	0	0	0	0	5 (1.0)	0	10 (1.5)	5 (1.2)
Hyperkeratosis	Incidence	P	0	0	1 (2.0)	0	5 (1.0)		18 (1.4)	7 (1.0)
Submucosal inflam.	Incidence	P	0	0	0	0	2 (1.0)	0	4 (1.3)	2 (2.5)
Submucosal oedema	Incidence	P	0	0	0	0	1 (1.0)	0	2 (2.0)	1 (2.0)

n.s.f.: no specific findings * (mean severity);

#: one high-dosed female showed lethargy, diarrhea and dystocia and had cannibalised its pups

Table Summary of reproductive performance (corresponds to the lower part of Table A6_8_2-2 MBO Doc IIIA 6.8.2_1.)

Parameter		Generation	control		low dose		medium dose		high dose	
			m	f	m	f	m	f	m	f
Reproductive Performance										
Implantations	Group means	P		12.8		13.0		13.3		13.1
Post implantation loss	% of implantations	P		7.5		12.3		14.7		16.3
	Litters affected	F1		10		12		14		10
	Total	F1		20		35*		41**		47**
	Mean	F1		1.0		1.6		2.0		2.1
	N	F1		21		22		21		22
Post natal loss (days 0-4 p.p.)	% of living pups	F1		10.9		4.0		2.9		7.9
	Litters affected	F1		4		8		3		5
	Total	F1		27		10**		7**		19
	Mean	F1		1.3		0.5		0.3		0.9
	N	F1		21		22		21		22
Dead pups at first litter check	Litters affected	F1		3		1		5		6
	Total	F1		3		2		12		24
	Mean	F1		0.1		0.1		0.6		1.1
	N	F1		21		22		21		22

* or **: Fisher's Exact test at 5% or 1% level, respectively

The CLH report stated that in addition to local stomach effects the sum of post-implantation loss and post-natal loss was increased in females at 45 mg/kg bw and concluded that there were 'no effects sufficient for classification for reproductive toxicity'.

The study authors interpreted the implantation loss and reduced pup survival at the high dose, 45 mg/kg bw/d, as substance-related effects. Although implantation losses were also seen at the low and mid dose levels and the pup viability was reduced at the mid and high dose level, these findings were not considered by the study authors to be treatment related and therefore they established the NOAEL for reproduction/developmental toxicity at 15 mg/kg bw/d.

The CLH report reported the increased implantation losses and the increased incidences of pup death only as a sum (without giving further quantitative information). Justification for the lack of more detailed documentation of these effects was included in a separate assessment document (MBO Doc III A 6.8.2-2) in which it was argued that it could not be determined whether there had been post implantation loss or post natal loss, where pups were cannibalised directly after birth by its mother. The author of the assessment document found it

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prudent to calculate the number of post implantation losses plus the number of postnatal losses between Day 0 and 4 as the combined incidence based on separate incidences and to reflect only on this combined incidence for the overall conclusion. The overall numbers of the sum were reported as 47, 45, 48, 66 in the control and dose groups.

In addition, the individual study findings were summarised by the RMS in a table (Table 4 of the MBO DocIII A 6.8.2-2) indicating that numbers of pups could not be reported for 2 of 24 dams. This means that the numbers of living animals after birth and at Day 4 are available for 22 dams (as documented in the Table A6_8_2-2 (see above)).

The result of considering solely the combined incidences is that a treatment-related effect became obvious only at the high dose where forestomach ulcerations were seen in some females. The (applicant's) assessment concluded that the number of implantation losses and increased pup mortality were effects of maternal toxicity.

Table: Summary of implantation loss and pup viability
(extracted from MBO Doc III A 6.8.2-1 and 6.8.2-2)

Effects	Groups	Control	5 mg/kg bw/d	15 mg/kd bw/d	45 mg/kg bw/d
Litters		21	22	21	22
Implantation sites		268	285	279	288
No. of implantation/litter		12.8	13.0	13.3	13.1
Implantation loss/number§		20	35*	41**	47**
Implantation loss/ %		7.5	12.3	14.7	16.5
Early implantation losses/litter		0.8	1.6	1.4	1.1
§Thereof: Dead pups at first litter check (Day 0/1)		3	2	12	24
Dead pups/litter (Day 0/1)		0.1	0.1	0.6	1.1
Live pups at Day 0/1		248	250	238	241
Post-natal loss Day 0-4 (%)		27# (expected 2.7) (10.9)	10** (4.0)	7** (2.9)	19 (7.8)
Total dead pups/litter (Day 0-4)		1.4 (expected 0.2)	0.5	0.9	2.0
Live pups at Day 4		221 (expected 242.3)	240	231	222
% of live pups related to numbers of implantation site		82.5 (expected 90.4)	84.2	82.3	77

Abnormally high spontaneous pup deaths until Day 4, expected value based on historical control data on mean survival of 98.9%

* / **: Fisher's Exact Test significant at 5% (*) or 1% (**) level

The RAC summarises the outcome of the 1-generation study as follows:

- There is no indication from the 1-generation study on RP 3:2 that male fertility was affected. There is also no evidence from the 1-generation study for an effect on fertility with regards to the number of implantations per litter.
- There is no indication of severe systemic toxicity in the females during and at the end of the treatment period. The slightly reduced food consumption of high dose females during the last gestational week only (-6.5%) did not affect the body weight. In high dose male rats at the end of the pre-pairing period (day 70), the food consumption was slightly lower (-5.5%) than in controls.
- The total numbers of implantation loss and the implantation losses per litter were increased in the low, mid and high dose groups in comparison with the control incidence. However no clear dose-response was observed.
- In 10/22 dams of the high dose group implantation losses were seen. Only in 4 of 24 dams that received the high dose (in 3 of 22 evaluated high dose dams; 2 of initially

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treated 24 dams cannibalised their pups and were therefore not considered for evaluation, one of these showed a moderate ulceration of the stomach (No. 185)) paired female rats ulcerations of the forestomach were reported. Its severity is reported as moderate to marked (the information on the grading is lacking in the CLH report and the summary Table A6_8_2-2, but can be found in MBO Doc III A 6.8.2-2 p.7ff and in the study report). Less severe stomach effects such as squamous hyperplasia (in 5 rats), hyperkeratosis (in 7 rats) and submucosal inflammation (in 2 rats) reported as minimal to moderate, were also observed in dams of this dose group.

- The 2 high dose dams (No. 178, 185) that cannibalised their pups were not included in the evaluation of the reproduction parameters.
- Whether the increases in implantation losses and dead pups occurred in litters from dams with forestomach lesions, is not documented in the CLH report. The RMS analysed individual findings (Page 7ff of MBO Doc III A 6.8.2-2): 4 of 24 pregnant females had ulcerations of the forestomach. The implantation losses in three of them were 1 of 8 implantation sites, 0 of 14 implantation sites and 13 of 15 implantation sites (Animal no. 179, 180, 190 of 22 dams evaluated). One dam (no. 185) with ulceration cannibalised its pups (not considered in the evaluation, Table A6_8_2-2). Regarding the implantation losses, the historical control data in the Harlan laboratories were reported from 17 studies with a median of 7.9% (range 1.6% to 12.2%, no data from the years when the studies were conducted). The control group in the 1-generation study corresponded well to the median value of the historical controls, meaning that 0.96 implantation losses per litter may be considered normal. Considering the number of dams with 2 or more implantation losses out of 22 dams there was no effect on the stomach in 4 dams with increased implantation losses (no. 169, 175, 184, 192) and minor effects such as hyperkeratosis and/or squamous hyperplasia (effects that primarily protect against irritation) were seen in two dams with increased implantation losses (no. 173, 176). In conclusion, increased implantation losses were seen in dams without effects on the forestomach, in dams with slight effects in the forestomach and in 1 out of 4 dams with ulceration of the forestomach. This distribution does not support the conclusion that severe (ulcerative) effects of the forestomach were causally related to the implantation losses.
- In the low and mid dose groups the implantation losses occurred without *any* effect on the stomach or *any* other sign of systemic toxicity. However, considering the lack of a dose response, the overall small increase of losses per litter in comparison to the control level and the observation of the highest loss per litter in the low dose calls a treatment-relationship for this effect in question.
- Pup survival at the first litter check (Day 0/1) was dose-dependently reduced in the mid and high dose groups. No indication of maternal toxicity was seen in the mid dose dams (the incidences of stomach lesions in high dose dams see above). The total number of dead pups on Day 0/1 were 3 (in 21 control litters), 2/22, 12/21 and 24/22 in the low, mid and high dose groups, respectively. The numbers correspond to 0.1, 0.1, 0.6 and 1.1 animals per litter, respectively.
- Based on the observed numbers of deaths no treatment-related effect on pup survival from Day 1-4 was observed. A dose-response relationship for this observation period was less clear. Increased post-natal losses were seen in the control and high dose groups; losses were significantly lower than in control animals from Day 1-4 deaths in the mid and low dose groups. An unusually high rate of pup death was seen in the control groups due to the high numbers of mortalities in 3 litters, mainly on Day 2. This finding may have caused the low death rates at the low and mid dose levels and may have masked a treatment-related effect at the high dose, but these assumptions could not be verified as no causalities were identified. Thus, effects on pup survival on Day 0-4 were uncertain and were not taken into account for classification purposes.

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Finally, the only fertility-related effect which was possibly related to the treatment was the reduced pup survival at first litter check (Day 0/1).

Other information from repeated dose studies:

- There was no indication from the repeated oral toxicity study on RP 3:2 that male fertility was affected.

Developmental toxicity

A developmental toxicity study conducted according to OECD TG 414 rabbits were gavaged with 0, 5, 35, 90 or 135 mg/kg bw/d RP 3:2 in corn oil. A dose of 135 mg/kg bw/d resulted in severe maternal toxicity (decrease in food consumption (GD 11-14, GD 28), lower body weight (max. – 9%), reduction in bw increase (GD 9-12,18-21,27-29, no quantitative data) increased mortality (10 pregnant rabbits between GD 9-23, one non-pregnant rabbit at Day 17, 4 dams sacrificed after abortions).

Necropsy revealed local lesions in the stomach in 10 22 dams at 135 mg/kg bw/d and an increased incidence in dilatation of the renal pelvis (in 12 of 22 dams of this group). (No data on histopathology was available.) The authors suggested a NOAEL for maternal toxicity at 90 mg/kg bw/d. Reddening of the stomach occurred in 3 of 23 dams at 45 mg/kg bw/d and in 3 out of 21 dams at 90 mg/kg bw/d. The study authors interpreted the stomach effect as corn-oil induced effects which according to the RMS was not substantiated. No information was available to evaluate the note of the RMS that the food consumption and body weight was decreased in the control and treated groups. A dilatation of the renal pelvis was observed in 12 of 22 dams at 135 mg/kg bw/d and in 4 of 24 dams at 5 mg/kg, 4 of 23 dams at 45 mg/kg and in 4 of 21 dams at 90 mg/kg bw/d.

Total implantation loss was seen in 3 of 22 dams (N. 77, 80, 83) at 135 mg/kg bw/d, in one of 24 dams at 5 mg/kg bw/d (No. 30), one of 22 dams at 90 mg/kg bw/d (No. 120). Developmental toxicity findings such as an increased number of early and late resorptions, a decreased number of foetuses, an increase in post-implantation losses and mortality of foetuses was only observed at 135 mg/kg bw/d.

No increase in the incidence of retardations, variations or malformations was detected in any treatment group.

Total implantation loss seen in 3/22 dams were only seen at 135 mg/kg bw/d, a dose at which 10 dams showed detachment and reddening of the stomach mucosa, 12 dams had dilated renal pelvis and premature deaths of 11 dams were seen.

Table: Macroscopic finding with the dams (corresponds to MBO Doc A III 6.8.1_02, Table 6.8.2-3)

Macroscopic finding with the dams

Macroscopic findings	Control n = 23 #	5 mg/kg n = 24 #	45 mg/kg n = 23#	90 mg/kg n = 21 #	135 mg/kg n = 22 #
Stomach:					
mucosa: detachment (and reddening)	1 #	2 #	1 #	0	10 #
reddened	0	0	3	3 #	10#
ulcers (a few, multiple)	0	2 #	1	0	4 #
haemorrhagic/dark/reddish/black	0	0	1	4 #	8 #
distension	0	0	0	0	3
Large Intestine:					
reddened	0	0	1 #	1 #	0
Duodenum:					

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reddened	0	0	0	0	2 #
Kidney:					
dilatation of renal pelvis	1	4 #	4 #	4	12 #
cysts (medulla, cortex)	0	1	1	0	0
coarse structure (cortex)	0	0	2	0	2 #
very soft	0	0	0	0	1 #
Spleen:					
reduced in size	0	0	1	3 #	1
Liver:					
pale (with dark foci)	0	0	6	3	2
glossy (light and dark)	0	0	0	0	1
Lungs:					
right lobe reddened	0	0	0	0	1 #
Vagina:					
reddish mucus	0	0	0	0	2

(including) prematurely deceased animal(s)

Table: Summary of animals examined (corresponds to MBO Doc A III 6.8.1_02, Table 6.8.1-1)

Summary of animals examined

	Group 1 Control	Group 2 5 mg/kg	Group 3 45 mg/kg	Group 4 135 mg/kg	Goup 5 90 mg/kg
Treated dams	24	24	24	24	24
Non-pregnant dams	1 ¹²	0	1 ¹²	1 ¹²	2
Dams with abortion	2	1	2	4	1
Dams without viable fetuses	0	1 ¹³	0	3 ¹³	1 ¹³
Prematurely deceased dams (pregnant and non-pregnant)	2 ¹²	2	2 ¹²	11 ¹²	3
Not examined dams	0	0	0	1 ¹⁴	1 ¹⁴
Evaluated litters	20	20	20	5	16

¹² The non-pregnant dams no. 9, 64 and 83 died prematurely.

¹³ A total post-implantation loss was noted in dams no. 30 (group 2), no. 77, 80 and 93 (group 4) and no. 120 (group 5).

¹⁴ Dams no. 86 and no. 114 injured their spine and were excluded from evaluation.

Assessment and comparison with the classification criteria

Formaldehyde

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

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

Effects on fertility

The available data from repeated dose toxicity studies and from a 1-generation study with 70 days of pre-mating treatment gave no indication of adverse effects on sexual function or fertility in male rats.

Based on the 1-generation study in rats it was concluded that RP 3:2 induced reduced pup survival at first litter check (Day 0/1) in the mid and high dose groups. Although the increase appeared to be dose related, the overall increase on a per litter basis was limited. This finding did not correspond to the effects on pup survival seen at Day 1-4, where no clear dose response was observed. The number of dead pups on Day 1-4 were higher at the low dose than at the mid dose and an unusual high numbers of pups died (mainly on Day 2/3) in the control groups. RAC questioned the reliability of the study and found the observed effects to be borderline and not sufficient to justify a classification for this endpoint.

In conclusion, in agreement with the DS' s proposal RAC agrees that a **classification of RP 3:2 for fertility is not warranted**.

Developmental toxicity

A developmental study conducted according to OECD TG 414 on rabbits gavaged with 0, 5, 35, 90 or 135 mg/kg bw/d RP 3:2 did not reveal adverse effects on the development or increased rates of malformations that require classification. A dose of 135 mg/kg bw/d resulted in severe maternal toxicity (decrease in body weight, increased mortality and abortions). Total implantation loss was observed in 3 dams out of 22 dams. The mortality rate in dams at this high dose was high; 11 dams out of 24 died prematurely. The CLP Regulation criteria states (in Annex I: 3.7.2.4.4) that data for a dose level should normally not be considered if mortality is excessive e.g. greater than 10%.

Based on the available data RAC concludes that **no classification for RP 3:2 is warranted for developmental effects**.

Thus, RAC concludes that no classification for reproductive toxicity is warranted for RP 3:2.

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, proprioceptive) 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. DocIII A6.4.1/02**) included functional observations and did not show respective specific effects. However the study is not considered as valid.

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4.11.1.2 Neurotoxicity – RP 3:2

In a subchronic rat study according to OECD guideline 408 summarized in **MBO-DocIII A6.4.1/02** 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 (\geq week 11) functional observations were recorded including sensory reactivity to different types of stimuli (auditory, visual, proprioceptive), 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/m ³ , but considered to be related to an unspecific irritation of the nasal/olfactory mucosa and their relevance to human health is unlikely

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.

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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 ¹H and ¹³C-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 D₂O 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 D₂O 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).

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Fig 5.1.1-1: Concentration and composition of hydrolysis products

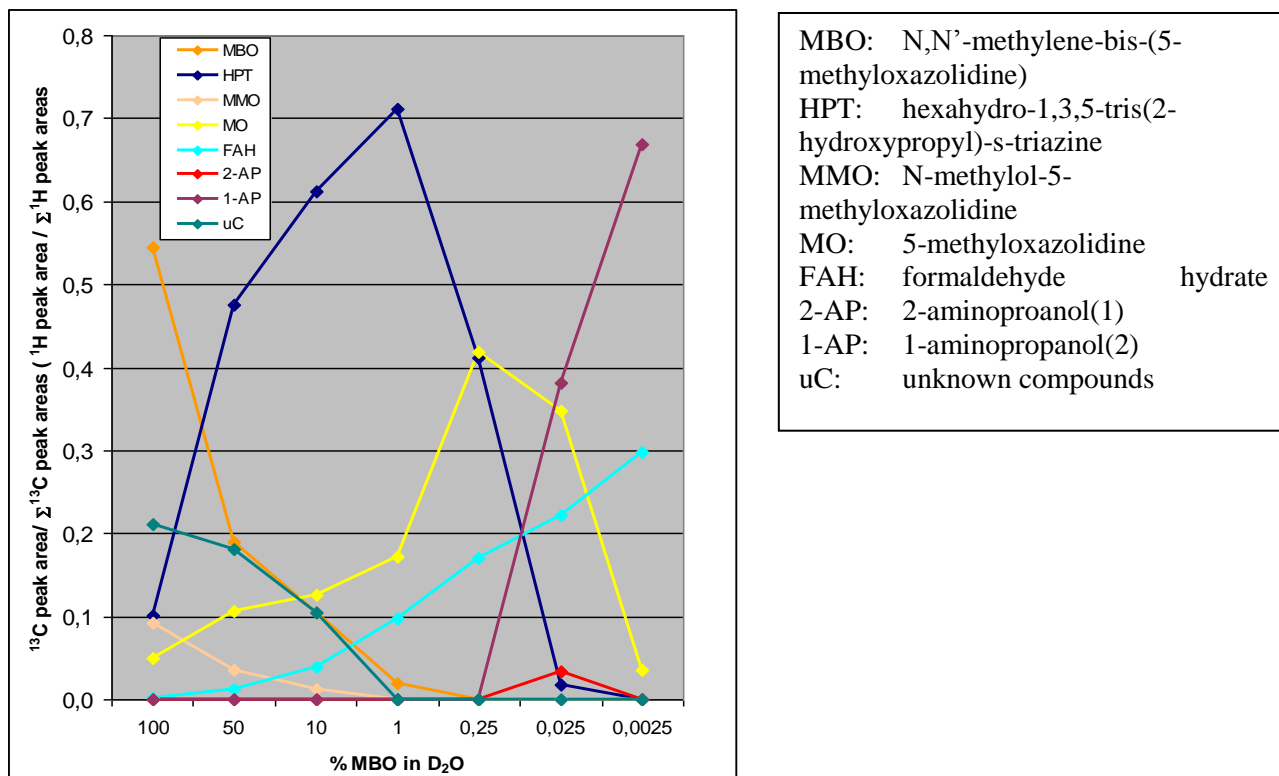


Table 5.1.1-2 Composition of hydrolysis products

Compound	Contram TM MBO in D ₂ O 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
Triazine (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-aminopropanol(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

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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 ₂ CO	time [h]	% H ₂ CO	time [h]	% H ₂ CO
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 DT₅₀ 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.

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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 ν	0 (expected)	-	-	Doc III-A 7.1.1.1.2 Justification for non-submission
Estimation indirect photolysis (Calculation AopWin v1.91)	OH	$3.13 \cdot 10^{-10}$ cm ³ /molecule s	$0.5 \cdot 10^6$ / 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 5×10^5 radicals per cm³ (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.

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Table 5.1.2-1 Biodegradation of the active substance

Guideline / Test method	Test type	Para- meter	Inoculum		Addition al substrate	Test substance concentr.	Degradation		Reference
			Type	Concen- tration			Incubatio n 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 A 7.1.1.1**) 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 A 7.1.1.2.2) was accepted based on the above mentioned arguments.

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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			Incubatio n 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_H CHO 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_H CHO 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_H CHO 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_H CHO 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

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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:

- $k = 1 \text{ h}^{-1}$ for biodegradation in sewage treatment plants;
- $k = 4.7 \times 10^{-2} \text{ days}^{-1}$ for surface water;
- $k = 2.3 \times 10^{-2} \text{ days}^{-1}$ 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 TM⁵ was run. The results indicate readily biodegradability.

A further QSAR calculation was performed with VEGA v1.0.8⁶ 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)⁷ 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)⁸.

⁵ <http://www.epa.gov/oppt/exposure/pubs/episuite.htm>

⁶ <http://vega.marionegri.it/wordpress/resources/qsar-in-silico-tools/>

⁷ 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

⁸ <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:%2278-96-6%22>, 2014-01-08

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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 are 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-hydroxypropylamine is susceptible to biodegradation, though study design and results vary. Overall, 2-hydroxypropylamine appears to be readily biodegradable.

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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)⁹ is 7.1 and an estimation of 2.75 was made by Environment & Health Canada, 2008¹⁰.

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

⁹ 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

¹⁰ http://www.ec.gc.ca/es-ees/CE2D78C6-9635-494E-9513-17D5D0C0223D/batch2_62-56-6_en.pdf

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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 m³ 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)¹¹ 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 $\log Kow$ range of 1 to 6. The BCF - $\log Kow$ 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”).

¹¹ 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

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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:

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/kg). 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 $\log K_{ow}$ range of 1 to 6. The BCF - $\log K_{ow}$ 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 $\log K_{ow}$ 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 $\log K_{ow}$ value and a DT50 hydrolysis of <1 hour.

In experimental studies on bioaccumulation no elevated formaldehyde levels were found. Additional information on $\log K_{ow}$ (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 K_{ow} value of -0.96 and a predicted BCF of 3.16 L/kg_{ww}. Moreover an experimentally determined BCF value from literature in the range of 2.7-3.6 L/kg_{ww} supports this finding.

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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 duration		Results [mg/L] ¹			Remarks	Reference
					LC ₀	LC ₅₀	LC ₁₀₀		
OECD 203 GLP, Klimisch 1	<i>Danio rerio</i> GrotaMar 71 [®]	Mortality	Semi-static	96 h	44.4	57.7 CI (51.5-64.6)	100	NOEC 29.63 mg/L	MBO - Doc III A7.4.1.1/0 1
OECD 203 GLP, Klimisch 1	<i>Danio rerio</i> Contram TM MBO*	Mortality	Semi-static	96 h	50	71 (geometric mean)	100	--	MBO - Doc III A7.4.1.1/0 2
OECD 203 No GLP, Klimisch 2	<i>Scophthalmus maximus</i> GrotaMar 71 [®]	Mortality	Semi-static	96 h	100	135	180	No monitoring of test subst.	MBO - Doc III A7.4.1.1/0 3

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 ContramTM 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 formaldehyde)). Observation in the first study

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(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 LC₅₀ 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	<i>Daphnia magna</i> Contram TM MBO*	Mobility	static	48 h		28	40	MBO - Doc III A7.4.1.2/01
OECD 202/I GLP, Klimisch 1	<i>Daphnia magna</i> Straus Grotamar 71 [®]	Mobility	semi- static	48 h	17.2	37.9 (CI 35 – 42)	55.6	MBO - Doc III A7.4.1.2/02
ISO/TC147 /SC5/WG2/ N61 (draft) No GLP, Klimisch 3	<i>Acartia tonsa</i> 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-EC₅₀ value of 28 mg/l (geometric mean between two concentrations causing 0% and 100% mortality), while in the semi-static system a 48 h-EC₅₀ 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 EC₅₀ 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.

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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	<i>Daphnia magna</i> 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 (EC₁₀ 1.1 mg/L CI 0-3 mg/L; EC₅₀ 26.4 mg/L, CI 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 _r C	E _b C ₅₀ ¹	E _r C ₅₀ ²	
OECD 201 GLP Klimisch 3	<i>Desmodemus subspicatus</i> Contram TM 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	<i>Desmodemus subspicatus</i> 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	<i>Skeletonema costatum</i> (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

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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_rC_{50} 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

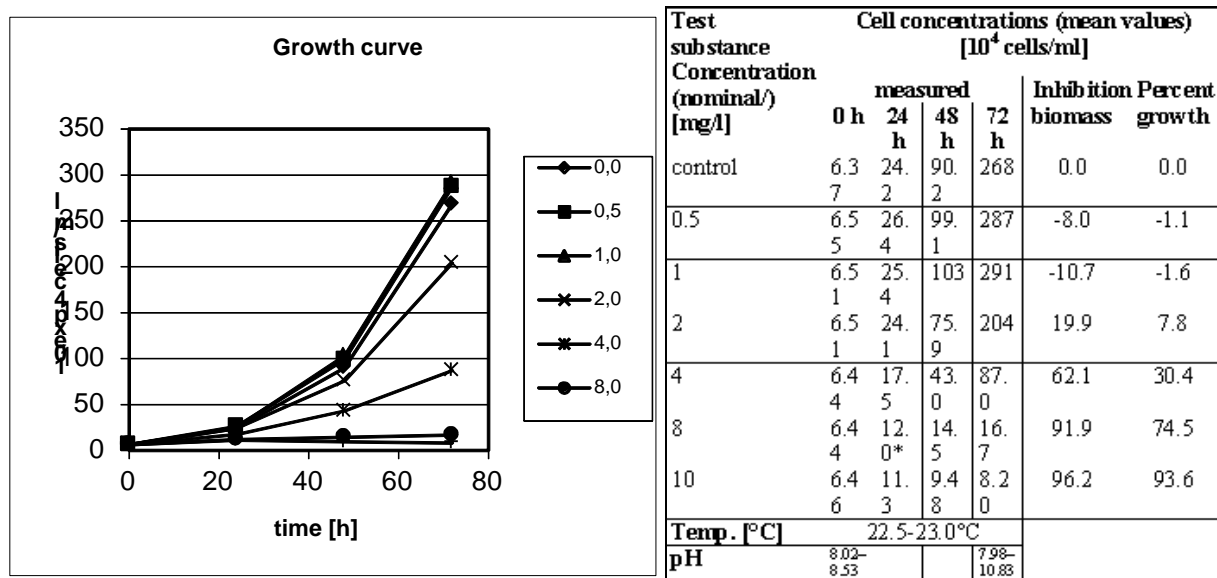
Test Solution	pH at t_0	pH at t_{72h}
Control with algal inoculation	8.53	10.83
Control without algal inoculation	8.50	8.22
10 mg/L with algal inoculation	8.46	8.15
10 mg/L without algal inoculation	8.44	8.09
8 mg/L with algal inoculation	8.51	8.12
8 mg/L without algal inoculation	8.39	8.07
4 mg/L with 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

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According to OECD (2000)¹² growth of algal test cultures can cause increase of pH due to consumption of HCO₃ 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)¹³ increased pH (≥ 8) facilitates penetration into green algae cells (*Scenedesmus obliquus*) of Methylamine (pKa=10.6, SRC PhysProp Database)¹⁴, 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)¹⁵ 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 t₀ and t₇₂. 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)



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_rC₅₀ of 3.77 mg/L (CI

¹² 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

¹³ Abeliovich A, Azov Y. 1976: Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol. Jun;31(6):801–806

¹⁴ <http://esc.syrres.com/fatepointer/webprop.asp?CAS=74895>, 2013-12-12

¹⁵ 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

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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 strengthened 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 design	duration	Results [mg/L]			Reference
						NOEC	EC_{50}	EC_{80}	
OECD 209 No GLP, Klimisch 2	Contram TM 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 $PNEC_{\text{microorganisms}}$ 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 EC_{50} of 44 mg/L it is clearly showed that no adaption of the inoculum occurred. The GLP study is much better reported

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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)C₅₀ values for all three trophic levels >1 mg/L;

Lowest L(E)C₅₀ value: E_rC₅₀ (algae) =2.4 mg/L

→ **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. -> **LC₅₀ (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]” -> **EC₅₀ (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_rC₅₀ (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 NOEC 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.

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Aquatic Chronic 1:

→ 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. -> **LC₅₀ (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]” -> **EC₅₀ (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_rC₅₀ (algae) = 0.5 mg/L,**

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

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

The substance “Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 3:2)” (RP 3:2) is a formaldehyde-releasing UVCB substance with bactericidal and fungicidal properties and is employed as a biocidal active substance.

Degradation

The dossier submitter proposed to consider RP 3:2 as rapidly degradable. The basis for this proposal was that although Contram™ MBO and GrotaMar 71 is proven to be not readily biodegradable, it is demonstrated that for RP 3:2 primary abiotic degradation via hydrolysis in the aquatic environment has a half-life <16 days (corresponding to a degradation of >70 % within 28 days) and the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Aquatic Bioaccumulation

According to the dossier submitter, RP 3:2 does not meet the CLP criteria for bioaccumulation. There are no experimental data on bioaccumulation of RP 3:2 available, however, based on the hydrolysis products formaldehyde (logK_{ow} = 0.48) and 2-hydroxypropylamine (logK_{ow}=0.61) the potential for bioaccumulation of RP 3:2 was considered low.

Acute Toxicity

The dossier submitter proposed to not classify RP 3:2 as acutely hazardous to the aquatic environment. The basis for this proposal is that L(E)C₅₀ values for all three trophic levels are >1 mg/L and the lowest L(E)C₅₀ value was derived for algae with E_rC₅₀ = 2.4 mg/L.

Chronic Toxicity

The dossier submitter proposed to classify RP 3:2 as Aquatic Chronic 3 (H412) based on rapid degradability and the lowest chronic toxicity in algae (*Desmodemus subspicatus*) with a NOE_rC of 0.5 mg/L. Algae have been shown to be the most sensitive species in aquatic acute toxicity tests. Two studies with the same species showed comparable results (E_rC₅₀ of 2.4 and 5.7 mg/L) and a third study on marine algae confirmed the findings from the freshwater algae tests (E_rC₅₀ of 3.8 mg/L). The chronic Daphnia study according to OECD Guideline 211 resulted a NOEC of 1.3 mg/L based on reproduction. For fish no chronic study was available.

Comments received during public consultation Three MSCA commented the ENV part of the classification dossier.

One commenting MSCA questioned the scientific significance of the information on hydrolysis rate and considered that further information is needed to verify the conclusion on hydrolysis. Another MSCA highlighted that for the two hydrolysis degradation products 2-hydroxypropylamine and formaldehyde it is unclear if additional data for an environmental hazard classification are available. One commenting MSCA requested a new algae study.

Assessment and comparison with the classification criteria Degradation

RAC notes that degradability tests on UVCB substances may only be considered relevant if it has been shown that the UVCB substance only contains structurally similar constituents that are expected to behave in the same way in the tests. RAC in general prefers the assessment of degradation via a testing approach where relevant constituents of a UVCB substance are first subjected to screening assessment individually. If certain constituents represent the worst case with regard to degradability, these "defined constituents" may be used for further testing and for assessing the entire UVCB substance.

Ready biodegradability

RAC notes that RP 3:2 (ContramTM MBO and Grotamar 71) has proven in two tests according to OECD TG 301D (Closed-Bottle-Test) to be not readily biodegradable. It was also shown that RP 3:2 was not toxic to the microorganisms. Both hydrolysis products formaldehyde and 2-hydroxypropylamine alone on their own. Consequently, the observation that the UVCB substance RP 3:2 is not readily degradable may indicate that it might include constituents which biodegrade at a slower rate or biodegradation products are formed which degrade more slowly. The fact that the 10-day window is not fulfilled might be an indication. Overall no further explanation of the negative test result is given which would question the reliability of the study for classification purposes.

RAC also takes note of a test on biodegradation in seawater according to the OECD TG 306 (Closed-Bottle-Method) with two test concentrations tested. In the higher test concentration only 54% degradation was observed after 28 days supporting the negative test results from the TG 301D tests. Only in the lower test concentration a degradation of 69% seem to indicate a limited potential for primary degradation but may not be used as indication of ultimate degradation of all constituents of the UVCB-substance RP 3:2.

RP 3:2, being an UVCB-substance, might contain constituents which are not sufficiently similar with regard to the property tested. Consequently, the degree of ultimate degradation (mineralisation to CO₂) of each of the various constituents and degradation products remains unknown in standard screening tests such as OECD 301D. It seems not possible to calculate a ThOD for RP 3:2 and a more careful consideration of the nitrification is recommended when measuring the COD. It is known that ready biodegradability tests may sometimes fail because of the stringent test conditions and that consistent positive test results should generally supersede negative test results. However, in the case of the UVCB substance RP 3:2 the positive test result of the TG 306 test in only one concentration may not be evaluated as a consistent positive test result superseding the negative test results from two 301D tests and one test concentration in the 306 test.

In a weight of evidence approach, taking all available information into account, RAC concludes that the UVCB-substance RP 3:2 is not readily biodegradable. This is in line with the assessment of the UVCB-substance RP 1:1.

Hydrolysis

It has been demonstrated in a laboratory test that RP 3:2 hydrolyses to formaldehyde and 2-hydroxypropylamine at rather low concentrations within a few hours. RAC notes that hydrolysis of RP 3:2 is rather the establishment of an equilibrium than irreversible hydrolysis. Consequently, the hydrolysis rate may not be taken as an abiotic degradation half-life as such. A more careful consideration of the hydrolysis is recommended. In addition, it has been demonstrated that hydrolysis of RP 3:2 is strongly dependent on its concentration in water and complete hydrolysis may only be assumed at very low concentrations. The CLP guidance requires that hydrolysis has to be demonstrated under relevant environmental conditions. Since RP 3:2 is a UVCB substance, degradation may not follow single first order kinetics. Both degradation rate independent from concentration and degradation following first order kinetics, are required to extrapolate laboratory results to relevant environmental conditions (see guidance IR R.7b). At the 32th meeting of ECHA's Member State Committee (MSC-32) it was agreed that relevant environmental conditions include 12°C temperature. Although, the hydrolysis half-life DT₅₀ under relevant environmental conditions (temperature, concentration, and pH) was not calculated, it may be reasonable to consider that the primary degradation half-life would be shorter than 16 days.

Rapid degradability

Following the guidance on the application of the CLP criteria (version 4.1, June 2015, II.2.3.8 Hydrolysis) *"only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment, data from hydrolysis studies could be considered."* While it has been demonstrated that RP 3:2 hydrolyses to the degradation products formaldehyde and 2-hydroxypropylamine, it was questioned in the PC if it has been sufficiently demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

RP 3:2 is a formaldehyde-releasing UVCB substance with bactericidal and fungicidal properties and it is scientifically well understood that the ecotoxicological properties are caused mainly by the hydrolysis product formaldehyde. Algae are the most sensitive species for the formaldehyde releaser RP 3:2, RP 1:1.

For the hydrolysis product formaldehyde, ecotoxicity data have been assessed during the Biocides Review Programme in 2012 ("Formaldehyde Core Dossier"). All three trophic levels, fish, invertebrates and algae have been tested for acute aquatic toxicity. The sensitivity is at the same level, i.e. around 5.7 mg/L and above the CLP criteria to classify

for aquatic acute hazard. However, no long-term study on fish is available. The algae study is only available as a literature publication without any raw data or concentration-response curves. Only the 72h E_rC_{50} of 5.7 mg/L was published. Consequently, the literature data does not allow the derivation of a NOE_rC , nor an E_rC_{10} or an E_rC_{20} and thus from this study no information on the chronic algae toxicity of formaldehyde is available. A second algae study was requested by several MSCA during the Biocides Review Programme and by one commenting MSCA during PC, but up to date has not been provided. For a *Daphnia* sp. a NOEC of 1.04 mg/L was derived, which is close to the criterion (<1 mg/L) for classification.

There is evidence that formaldehyde is slightly more toxic than RP 3:2. Acute toxicity data show that fish are up to 12 times and invertebrates up to 6 times more sensitive against formaldehyde than against RP 3:2, while the sensitivity of algae is nearly identical. The chronic toxicity data for invertebrates show a slightly higher sensitivity to formaldehyde.

In 2012 RAC adopted its opinion on the proposal submitted by France for a harmonised classification and labelling at EU level of formaldehyde. However, the endpoint and classification as hazardous to the aquatic environment were not part of the dossier and have not been evaluated by RAC.

For the second relevant hydrolysis product 2-hydroxypropylamine an OECD assessment dated 2011 summarises acute ecotox data and QSAR estimations for all three trophic levels. The available information seems to indicate that 2-hydroxypropylamine to not fulfil the CLP criteria for aquatic acute toxicity. However, none of the available information was considered to be a reliable key study by the dossier submitter. No additional information and none of the original study reports or scientific article were provided. Chronic toxicity of 2-hydroxypropylamine was not available in the CLH report. RAC concludes that the data do not sufficiently demonstrate that the hydrolysis product 2-hydroxypropylamine, does not fulfil the criteria for classification as hazardous to the aquatic environment.

The CLH report shows in Figure 5.1.1-1 that at least one other known degradation product and a number of unknown compounds may be formed by hydrolysis (depending on the initial concentrations) and no information on them is presented if they do not fulfil the criteria for classification as hazardous for the aquatic environment.

In summary, RAC considers RP 1:1 to be not ready biodegradable but hydrolysable. RAC agrees with the commenting MSCA that it has not been sufficiently demonstrated that the two relevant hydrolysis products (formaldehyde and 2-hydroxypropylamine) and in particular other potential hydrolysis products do not fulfil the criteria for classification as hazardous to the aquatic environment. As consequence, RAC considers RP 1:1 to be not rapidly degradable for the purpose of classification.

Aquatic Bioaccumulation

RAC notes that a UVCB substance may only be considered to be one chemical substance for the purpose of assessing and testing the potential to bioaccumulate, if a clear case is made in the assessment for why all constituents are sufficiently similar with regard to the property tested. In the case of RP 3:2, RAC agrees with the dossier submitter that, although there are no experimental data about bioaccumulation available, in view of the rapid hydrolysis, it may be assumed that RP 3:2 does not fulfil the criteria on aquatic bioaccumulation.

Acute Toxicity

RAC agrees with the dossier submitter to not classify RP 3:2 as acutely hazardous to the

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aquatic environment.

Chronic Toxicity

RAC agrees with the dossier submitter that the lowest chronic toxicity of RP 3:2 was derived for algae with a NOE_{rC} of 0.5 mg/L. However, in contrast to the dossier submitter RAC considers RP 3:2 to be not rapidly degradable for the purpose of classification. This would result in a classification of RP 3:2 as Aquatic Chronic 2 (H411). RAC also applied the surrogate approach since adequate studies on chronic fish toxicity using RP 3:2 as the test substance were not available. The surrogate approach results (the substance not rapidly degradable and the fish LC₅₀ of 58 mg/L) in a classification of RP 3:2 as Aquatic Chronic 3 (H412). Since the most severe outcome is chosen, as indicated by the guidance, **RAC concludes that RP 3:2 should be classified as Aquatic Chronic 2 (H411).**

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 trophic levels L(E)C ₅₀ values > 1mg/L are available.
Signal words	-	
Classification	Aquatic Chronic 3	
Hazard statements	H412: Harmful to aquatic life with long lasting effects	Chronic Toxicity: Rapidly degradable substance for which adequate chronic toxicity data are available for daphnia and algae. Lowest chronic value is the NOE _{rC} s from algae with 0.5 mg/L -> Aquatic Chronic 3.
Precautionary Statements	General	
	Prevention	
	Response	
	Storage	
	Disposal	

7 OTHER INFORMATION

Not available

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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
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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 TM 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	¹³ C 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. Regelman, 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. Regelman, 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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Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
A3.1.1/02	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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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 – Aufklärung 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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A3.9/02	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 & Mayr 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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A4.2b	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
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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
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		20-4-0155-01-01 GLP, unpublished		Lubrizol
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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
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Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
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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, Modified 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, 25 th 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 maximisation 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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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
		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 Ltd., 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 Grotamar71 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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Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
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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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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
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 (<i>Brachydanio rerio</i>) (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 <i>Scophthalmus maximus</i> . 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 <i>Daphnia magna</i> 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
A7.4.1.2/03	1995	The Acute Toxicity of MAR 71 to the Marine Invertebrate <i>Acartia tonsa</i> . Acer Environmental Laboratories and Sciences, Study No. AE950217, Dec. 1995 Non GLP, unpublished	Y	Schülke & Mayr

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Section No / Reference No	Year	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
A7.4.1.3/01	2000	Study on the toxicity towards algae of “3,3’-Methylene-bis [5-methyl-oxazolidine]” according to OECD-Test Guideline 201 (“Alga, Growth Inhibition Test”). Institut Fresenius, Study No. IF-99/21380-00 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.3/02	1995	Testing of Toxic Effects of MAR 71 on the Single Cell Green Alga <i>Scenedesmus subspicatus</i> . Arbeitsgemeinschaft GAB Biotechnologie GmbH und IFU Umweltanalytik GmbH, Report No. 95103/02-AASs GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.3/03	1995	The Acute Toxicity of MAR 71 to the Marine Alga <i>Skeletonema costatum</i> . Acer Environmental Laboratories and Sciences, Study No. AE950216, Dec. 1995 Non GLP, unpublished	Y	Schülke & Mayr
A7.4.1.4/01	1999	Determination of Acute Toxicity of Products towards Bacteria. Institut Fresenius, Study No. 99TE113603 Non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.4/02	1992	Untersuchung zur Klärschlamm-Toxizität von MAR 71 nach OECD 209 (“Activated sludge, Respiration Inhibition Test”). Schülke & Mayr GmbH, Forschung und Entwicklung, April 1992 Non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.1.4/03	2001	Activated Sludge, Respiration Inhibition Test of Grota MAR 71. Jai Research Foundation, Study No. 3335, July 26, 2001 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A7.4.3.4	2007	Study on the Chronic Toxicity towards Daphnia of „Reaction Product of Para-formaldehyde with 2-Hydroxypropylamin (Relation 3:2)” according OECD-Guideline No. 211 (<i>Daphnia magna</i> Reproduction Test). SGS Institut Fresenius GmbH, Study No. IF-07/00857685 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
Chapter 5	1997	Environmental Assessment of the Alkanolamines. Reviews of Environmental Contamination and Toxicology, Vol. 149. pp 87-137 GLP not applicable, published	N	-

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Chapter 5	ECHA 2012	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	N	-
Chapter 5	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	N	-
Chapter 5	Abeliovich A, Azov Y.	1976	Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol. Jun;31(6):801–806	N	-
Chapter 5	Bridie AL, Wolff CJM & Winter	1979a	BOD and COD of some petrochemicals Original; Water Res 13: 627-30 (1979)	N	-
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