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 1:1)

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 1:1)
EC number:	Not applicable
CAS number:	Not applicable
Annex VI Index number:	Not allocated
Degree of purity:	Please see text below
Impurities:	Please see text below

The biocidal active substance “Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)” (short: RP 1:1) is a complex mixture prepared by reaction of paraformaldehyde and 2-hydroxy-propylamine.

In a first step formaldehyde reacts with the NH_2-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 independant of the molar ratio of the starting materials to HPT or MBO. At a molar ratio paraformaldehyde / 2-hydroxypropylamine = 1:1 α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (HPT) is formed, while at a molar ratio of 3:2 with the aid of vacuum and energy mainly N,N’-methylene-bis(5-methyloxazolidine) (MBO) is the product. The reaction scheme is presented in Doc IIA confidential in Figure 1.2-1.

During production of the active substance, via an intermediate and subsequent elimination of water α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol is formed. The intermediate may be present as “open structure” (1-[hydroxymethylamino]propan-2-ol) or as ring structure (5-methyloxazolidine). The triazin ring seems to be thermodynamically more stable than the oxazolidine ring. The product includes water which was eliminated during synthesis.

The active substance (reaction product) is applied exclusively in aqueous solutions, where the substance is hydrolysed (cf. Doc II chapter 4). As in aqueous solutions formaldehyde is hydrated, the equilibrium is shifted towards the starting materials. The content of all constituents depends on the concentration of the active substance, the temperature and the pH-value. Because of hydrolysis, chromatographical methods or derivatization are not applicable to determine the content of the single constituents.

As an UVCB substance, the active substance is identified by its source and the manufacturing process (e.g., ratio paraformaldehyde and 2-hydroxypropylamine = 1:1, temperature, etc.). The starting materials are paraformaldehyde and 2-hydroxypropylamine, and the process is as given above (please also cf. to Doc. II-A confidential).

In addition, the active substance is specified by the main identifier “content of releasable formaldehyde”, which is typically 28%. The formaldehyde content in 10 measured batches (**Study A 2.7/03** and **A 2.7/04**)

has a range of 27.3%w/w to 28.4%w/w (mean value ± 3 x standard deviation). The company indicates a range of 26 – 30%w/w.

During product control (release) the reaction mixture is specified by its formaldehyde content and selected physical chemical properties (**Study A 2.7/01** and **A 2.7/02**)

Furthermore, NMR spectra can be used as fingerprint (qualitative) in order to identify the mixture. The composition and identity of the active substance (reaction mixture) was studied by ^{1}H and ^{13}C-NMR spectroscopy. Besides the signals from the main ingredient HPT, signals from hydrolysis products were observed. ^{13}C-NMR spectra are presented in Doc. II-A confidential, chapter 1.1. Batch analyses by NMR were performed (totally 5 batches analysed, 2 and 3 batches from production site 1 and 2, respectively) to show comparable composition of both products (Grotan® WS and CONTRAM™ 121) and to control the main constituents of each manufacturing plant. Comparison of the NMR spectra (Fingerprint) of the active substance manufactured at different plants as well as comparison of different batches shows a comparable composition (**Study A2.7/05**).

At least two production sites exist in Europe, located in Norderstedt and Hamburg, Germany. The biocidal products on the market are at least two products named Grotan® WS and CONTRAM™ 121, which are the active substances as manufactured. (For discussion of comparable composition of Grotan® WS and CONTRAM™ 121, please see Doc. II-A confidential.) However, in case active substances from other sources than specified in this CAR are intended to be used, technical equivalence to the reference source specified in this CAR has to be proven in advance.

Supporting data can be 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 Grotan WS (**Study A 2.7/06**), see Table 1.1-1 in Doc IIA confidential. These values give only a rough estimation about the composition of the reaction mixture and the concentration of the minor constituents.

1.1.1 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. In addition, there is a main identifier “content of releasable formaldehyde”, which is typically 28%. The formaldehyde content in 10 measured batches (**Study A 2.7/03** and **A 2.7/04**) has a range of 27.3%w/w to 28.4%w/w (mean value ± 3 x standard deviation). The applicant indicates a range of 26 – 30%w/w.

There are no additives in the active substance as manufactured.

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)	

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

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

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

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

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

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

	CLP Regulation (including criteria according to 2nd ATP of CLP)
Formaldehyde	
Current opinion by RAC	Carc. 1B H350 Muta. 2 H341 Acute Tox. 3* H301 Acute Tox. 3* H311 Acute Tox. 3* H331 Skin Corr. 1B H314 Skin Sens. 1 H317 Specific Conc. Limits: * Skin Corr.1B; H314: C ≥ 25 % Skin Irrit. 2; H315: 5 % ≤ C < 25 % Eye Irrit. 2; H319: 5 % ≤ C < 25 % STOT SE 3; H335: C ≥ 5 % Skin Sens. 1; H317: C ≥ 0.2 %
2-Hydroxypropylamine	
Current entry in Annex VI, CLP Regulation	Skin Corr. 1B, H314: Causes severe skin burns and eye damage

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
2.7.	Flammable solids	n.a.	n.a.	currently not classified	data lacking
2.8.	Self-reactive substances and mixtures	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	n.a.	n.a.	currently not classified	data lacking
2.10.	Pyrophoric solids	n.a.	n.a.	currently not classified	data lacking
2.11.	Self-heating substances and mixtures	n.a.	n.a.	currently not classified	data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	n.a.	n.a.	currently not classified	data lacking
2.15.	Organic peroxides	n.a.	n.a.	currently not classified	conclusive but not sufficient for classification

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

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

GHS Pictograms

Signal word: Danger

Hazard statements:
H314: Causes severe skin burns and eye damage
H317: May cause an allergic skin reaction
H350: May cause cancer
H341: Suspected of causing genetic defects
H412: Harmful to aquatic life with long lasting effects

Precautionary statements:
P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P273: Avoid release to the environment
P281: Use personal protective equipment as required
P260: Do not breathe mist/vapours/ spray.
P264: Wash ... thoroughly after handling.
P301 + P330 + P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 +P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313: IF exposed or concerned: Get medical advice/ attention.
P363: Wash contaminated clothing before reuse.
P310: Immediately call a POISON CENTER or doctor/physician.
P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.
P405: Store locked up.
P501: Dispose of contents/container to …

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

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

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

2.2 Short summary of the scientific justification for the CLH proposal

Human Toxicology:

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

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

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

Environment:

Acute aquatic toxicity: $L(E)C_{50}$ values between 1 - 130 mg/L; lowest acute value E_rC_{50} (algae) =2.9 mg/L;

Chronic Aquatic toxicity: lowest E_rC_{10} for algae = 0.148 mg/L, NOEC daphnia (read across) = 1.3 mg/L

Fate & behavior: 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 E_rC_{10} value from algae with 0.148 mg/L in combination with rapidly degradable.

2.3 Current harmonised classification and labelling

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

No current classification and labelling.

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

No current classification and labelling.

2.4 Current self-classification and labelling

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

No current classification and labelling.

2.4.2 Current self-classification and labelling based on DSD criteria

Classification	By the manufacturer
Class of danger	
R phrases	
S phrases	

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

Please see Part A, Chapter 1.

1.2 Composition of the substance

Please see Part A, Chapter 1 and Annex Doc. II-A confidential

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 CONTRAM™ 121 and Grotan® WS

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico - chemical properties

Property	Method	Purity/Specification	Results	Reference
Melting point	OECD guideline 102	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	<-30°C; no endothermic signals recognizable between -30°C and +30°C	**Doc. III-A 3; Study A3.1.1/01**

Property	Method	Purity/Specification	Results	Reference
	EEC A.1	Grotan® WS Purity: UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	-36°C to -38°C	**Doc. III-A 3; Study A3.1.1/02**
Boiling point	OECD guideline 103	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	Two endothermic signals were found with onset temperatures at 62.0°C and 148.8°C. This fact indicates that the test is not a pure compound. So no exact boiling point for the test item can be specified. Study technically not feasible (UVCB substance)	**Doc. III-A 3; Study A3.1.02/01**
	EEC A.2	Grotan® WS Purity: UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	The boiling point Grotan® WS is 110.03°C	**Doc. III-A 3; Study A3.1.02/02**
Density	OECD guideline 109	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	The relative density is D^{20}_{4}=1.0867±0.29 g/cm^3	**Doc. III-A 3; Study A3.1.3/01**
	EEC A.3	Grotan® WS Purity: UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	The relative density is D^{20}_{4}=1.11 g/cm^3	**Doc. III-A 3; Study A3.1.3/01**
	DIN 51757 D	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 100522411	The density is 1.0810 g/cm3. This is not the relative density.	**Doc. III-A 3; Study A3.1.3**

Property	Method	Purity/Specification	Results	Reference
Vapour pressure	OECD guideline 104	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	$6.4x10^{-5}$ Pa (20°C); $1.3x10^{-4}$ (25°C); 3.9 10^{-3} (50°C) The UVCB substance is unstable; probably hydrolysis products were measured in the gas phase. Test substance was degassed at 80±5°C and ca. 10-5 hPa for 18 hours prior to test.	**Doc. III-A 3; Study A3.2/01**
	EEC A.4	Grotan® WS Purity: UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	9.303 $x10^{2}$ Pa (25°C) for the unstable UVCB substance The UVCB substance is unstable; probably hydrolysis products were measured in the gas phase.	**Doc. III-A 3; Study A3.2/02**
	Epi Suite 3.12	Purity/Specification: α, α', α''-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (main constituent)	4.69 $x10^{-7}$ Pa (Calculation Epi Suite 3.12) The calculation is based on the main constituent, not on the UVCB substance.	**Doc. III-A 3; Study A3.2/03**
Henry´s Law Constant	Calculation based on QSAR	Purity/Specification: α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (main constituent)	2.55 $x10^{-6}$ Pa xm^{3} x mol^{-1} (25°C) (Calculation EPIWIN 3.12) The calculation is based on the main constituent, not on the UVCB substance.	**Doc. III-A 3; Study A3.2/02**
Physical state	Visual inspection	n.a. (visual inspection)	liquid	**Company Statement**
Colour	Visual inspection	n.a. (visual inspection)	Colourless to yellow	**Company Statement**
Odour	Olfactory inspection	n.a.	Amine-like	**Company Statement**
Absorption spectra: UV/VIS	Spectralphotometric determination	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.100341495	There are no absorption maxima >200 nm.	**Doc. III-A 3; Study A3.4/01**
	Spectralphotometric determination	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1119141	There are no absorption maxima >250 nm.	**Doc. III-A 3; Study A3.4/02**

Property	Method	Purity/Specification	Results	Reference
Absorption spectra: IR	Spectralphotometric determination	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 100522411	IR- spectra in agreement with proposed structure	**Doc. III-A 3; Study A3.4/03**
	Spectralphotometric determination	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1102591	IR- spectra in agreement with proposed structure	**Doc. III-A 3; Study A3.4/04**
Absorption spectra: NMR	^{1}H and ^{13}C-NMR	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 100499464	^{1}H, ^{13}C-NMR spectra in agreement with proposed structure.	**Doc. III-A 3; Study A3.4/06**
	^{1}H and ^{13}C-NMR	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1048125	^{1}H, ^{13}C-NMR spectra in agreement with proposed structure.	**Doc. III-A 3; Study A3.4/05**
Absorption spectra: MS	El-MS	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.100527798	Mass spectra in agreement with proposed structure.	**Doc. III-A 3; Study A3.4/07**
	VG Autospecsectorfield masspectrometer	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1048125	Mass spectra in agreement with proposed structure.	**Doc. III-A 3; Study A3.4/05**
Water solubility	OECD guideline 105 Flask - Method	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	Miscible with buffer solution at pH 5; 7.and 9 (20°C)	**Doc. III-A 3; Study A3.5/01**

Property	Method	Purity/Specification	Results	Reference
	OECD guideline 105 Flask - Method	Grotan® WS Purity: UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	Grotan® WS is miscible with water.	**Doc. III-A 3; Study A3.5/02**
Dissociation constant	n.a.	n.a.	The active substance HPT is a complex reaction mixture (UVCB Substance) intended to release formaldehyde in aqueous solutions. In aqueous solutions a dynamic equilibrium is formed of which the composition depends on the concentration, pH value and temperature The pH value of a 0.2% aqueous solution is 10.23 indicating basic properties of nitrogen containing constituents. (α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol cannot be isolated and therefore determinations of the single pKa values are not possible.	**Doc. III-A 3; Justification** **Doc. III-A 3; Study A3.6**
Solubility in organic solvents, including the effects of temperature on stability	OECD guideline 116	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	miscible with standard fat (37°C)	**Doc. III-A 3; Study A3.7/01**
	Visual inspection turbidity	Contram ™ 121: Purity/Specification active substance as manufactured (UVCB substance) Batch no. 24774	Completely miscible with DMSO, ethanol, n-octanol and aceton (21°C-23°C). Insoluble in toluene and cyclohexane (21-23°C)	**Doc. III-A 3; Study A3.7/01**
	Hach Method 8195(based on USEPA 180.1)	Contram ™ 121: Purity/Specification active substance as manufactured (UVCB substance) Batch no. 100502789	Solubility in heptane: 200-280 mg/L (21.7±0,5°C)	**Doc. III-A 3; Study A3.7/03**

Property	Method	Purity/Specification	Results	Reference
Stability in organic solvents used in b.p. and identity of relevant breakdown products	n.a.	n.a.	The active substance and the biocidal products are 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	OECD guideline 117 Calculation	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	based on formaldehyde:-0.4767±0.06 Based on isopropanolamine:-0.6108±0.04 The UVCB substance is unstable; only the hydrolysis products were measured. Total formaldehyde determined after derivatisation with hydroxylammonium chloride; total HPA determined potentiometrically. Result based on the sum of FA and HPA	**Doc. III-A 3; Study A3.9/01** **Doc. III-A 3; Study A3.5/02**
Thermal stability identity of relevant breakdown products	OECD guideline 113	Contram ™ 121: Purity/Specification active substance as manufactured (UVCB substance) Batch no. 24774	log Pow: endothermic effect at 40 -195°C; log Pow :exothermal effect at 195-260°C Endothermic effect could be caused by a slow transformation process forming volatile formaldehyde	**Doc. III-A 3; Study A 3.2/01**
	DSC screening test	TPI 1600 (Trimethyl-1,3,5-triazin-1,3,5-triethanol) (purity unknown)	log Pow exothermal effect from onset-temperature 160°C	**Doc. III-A 3; Study A 3.10/02**
Flammability, including autoflammability and identity of combustion products	EEC A.12	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	not flammable; no flammable gas was evolved and no ignition of the gas occurred	**Doc. III-A 3; Study A 3.11/01**
	EEC A.15	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	302°C at 102.1 kPa	**Doc. III-A 3; Study A 3.11/02**

Property	Method	Purity/Specification	Results	Reference
Flash point	EEC A.9	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	105 °C	**Doc. III-A 3; Study A 3.12**
Surface tension	OECD guideline 115	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1119141	69.1 mN/m. Grotan® WS is not surface active.	**Doc. III-A 3; Study A 3.13**
Viscosity	OECD guideline 114	Grotan® WS Purity/Specification: active substance as manufactured (UVCB substance) Batch no. 1100189	960 m x Pa x s at 20 °C	**Doc. III-A 3; Study A 3.14/01**
Explosive properties	n.a.	n.a.	The active substance is handled and marketed as aqueous solution, which prevents explosive properties. From the structural formula and the composition of the substance it can be safely concluded that the substance does not evolve any explosive properties.	**Doc. III-A 3; Justification**
Oxidizing properties	OPPTS 830.6314 EPA 712-C-96-023	Grotan® WS Purity/Specification UVCB substance (with Formaldehyde 26.4-28.0%w/w;2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	No reaction observed with water, mono ammonium; phosphate; potassium; permanganate and kerosene. The substance is not oxidising.	**Doc. III-A 3; Study A 3.16**
Reactivity towards container material	Company Statement		The biocidal product is packed and stored in LDPE containers or in steel barrels or containers coated with LDPE. Experience shows that these materials are suitable for storage and transport of the biocide.	Company Statement

2 MANUFACTURE AND USES

2.1 Manufacture

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

2.2 Identified uses

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

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

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

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

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

3.1 All hazard classes

3.1.1 Summary and discussion of all hazard classes

No classification is proposed based on available data.

3.1.2 Comparison with criteria

No classification is proposed based on available data.

3.1.3 Conclusions on classification and labelling

No classification is proposed based on available data.

4 HUMAN HEALTH HAZARD ASSESSMENT

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

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

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

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

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

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

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

Table 4-1 Conversion factors for reaction products with FA

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

This chapter shall serve as basis for concluding on the classification of RP 1:1. Data for RP 3:2, formaldehyde and 2-hyrdoxypropylamine are presented and discussed in parallel to support the conclusions for RP 1:1.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

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

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

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

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

Table 4.1-1 Toxicokinetics and metabolism of formaldehyde

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

Rate and extent of excretion	Metabolic elimination, high, but variable rate and extent of metabolite excretion (based on ^{14}C) mainly with air and urine (initial plasma t1/2 12 h, terminal t1/2 50 h, 10-40 % ^{14}C residues after 3-4 d)

4.1.3 Human information

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

4.1.4 Summary and discussion on toxicokinetics

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

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

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

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

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity – RP 1:1

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

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

Dermal	LD_{50} study OECD 402	Rat Wistar 5 m & 5 f	Limit test 2000 mg/kg bw undiluted test substance	Grotan WS Batch 1025145 FA 27.9%	LD_{50} > 2000 mg/kg bw in f and m (no mortality)	Incomplete data on local effects.	Schülke & Mayr (2000), DocIIIA6.1.2/02

f: females; m: males

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

4.2.1.2 Acute toxicity – RP 3:2

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

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

Dermal	OECD 402 GLP	Rat Wistar 5 m & 5 f	0, 1000, 1350, 1823 mg/kg bw undiluted substance,	GrotaMAR 71 Batch 1024828 FA 46.9% HPA 80,2%	LD50 = 1400 mg/kg bw for males and females combined mortality: 10/50/80% with increasing dose	≥ 1000mg/kg bw: Epidermal thickening/ erythema, scab	Schülke & Mayr (2000); DocIII A6.1.2/02
Dermal	OECD 402 GLP	Rat Sprague-Dawley 5 m & 5 f	250, 750, and 2000 mg/kg bw ~ 13%, 40% in corn oil and undiluted	Contram MBO Charge 24773 FA 42.28%	LD50 = 790 mg/kg bw for males and females combined; mortality: 1/10 animals in 750 mg/kg bw, 10/10 animals in 2000 mg/kg bw	With 2000 mg/kg bw erythema and oedema (all) and necrosis (2 animals) in high dose	Bode Chemie (2002); DocIII A6.1.2/03

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

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

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

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

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

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

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

		100% in 2000 mg/kg bw (neat a.s.) corrosive effects with undiluted substance	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 for RP 1:1 the acute toxic effects were secondary to corrosion. Classification of corrosive substances for acute toxicity is mechanistically redundant unless non-corrosive concentrations are tested. The latter is also a requirement of the respective OECD test guidelines. Therefore we propose no acute toxicity classification for the 3:2 and the 1:1 reaction product

4.2.5 Conclusions on classification and labelling

No classification is required.

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

RP 1:1 and RP 3:2 should be classified for corrosion, additional labeling for STOT SE 3 (respiratory irritation) would be redundant. Besides corrosive or irritant effects at the site of contact no other specific target organ toxicities are observed or expected.

Therefore no classification is required.

4.4 Irritation

4.4.1 Skin irritation

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

Not data are available.

4.4.1.2 Non-human information for RP 1:1

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

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

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

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

4.4.1.3 Non-human information for RP 3:2

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

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

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

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

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

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

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

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

4.4.1.5 Summary and discussion of skin irritation

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

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

4.4.1.6 Comparison with criteria

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

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

4.4.1.7 Conclusions on classification and labelling

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

4.4.2 Eye irritation

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

4.4.2.1 Non-human information for RP 1:1

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

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

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

4.4.2.2 Non-human information for RP 3:2

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

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

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

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

No human data available.

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

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

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

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

4.4.2.5 Summary and discussion of eye irritation

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

4.4.2.6 Comparison with criteria

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

4.4.2.7 Conclusions on classification and labelling

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

4.5 Corrosivity

See chapter 4.4

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information for RP 1:1

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

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

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

4.6.1.2 Non-human information for RP 3:2

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

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

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

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

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

4.6.1.3 Human information for RP 1:1

No human data available.

4.6.1.4 Human information for RP 3:2

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

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

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

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

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

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

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

4.6.1.6 Summary and discussion of skin sensitisation

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

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

4.6.1.7 Comparison with criteria

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

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

4.6.1.8 Conclusions on classification and labelling

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

4.6.2 Respiratory sensitisation

No data are available.

4.7 Repeated dose toxicity

4.7.1 Non-human information RP 1:1

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

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

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

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

4.7.2 Non-human information – RP 3:2

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

*: limited validity

4.7.5 Summary and Discussion of repeated dose toxicity

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

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

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

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

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

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

4.7.6 Comparison with criteria for STOT RE

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

No repeated dose inhalation studies with RP 1:1 or RP 3:2 are available. However based on the hydrolysis study and the toxicokinetic study it is plausible that by dilution by the reaction of formaldehyde with biological media the equilibrium mixture quickly shifts towards formaldehyde. Therefore the human data based local inhalative AEC of 0.12 mg/m³ for formaldehyde may be read across to MBM (on molar basis, factor 6.2) and used for assessing the risk from inhalation exposure (see Doc IIA3.12.1).

With repeated oral gavage dosing in rats and rabbits RP 1:1 and RP 3:2 as well as the hydrolysis product formaldehyde induced local effects at the site of contact, i.e. in the gastro-intestinal tract. The LOAELs were 80 mg/kg bw day and 60 mg/kg bw day. These LOAELs are within the guidance value range for STOT-RE 2 (oral, 10-100 mg/kg bw day). The LOAELs are also “more than half an order of magnitude lower that mediating the evident acute toxicity”, the oral LD50 (see chapter 3.9.2.5.1 in ECHA CLP guidance 2012).

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

4.7.7 Conclusions on classification and labelling for STOT RE

No classification necessary for STOT RE is required.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

4.8.1.1 In vitro data – RP 1:1

Table 4.8-1 RP 1:1 Genotoxicity in vitro

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

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

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

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

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

4.8.1.2 In vitro data – RP 3:2

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

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

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

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

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

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

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

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

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

MA: metabolic activation

4.8.1.4 In vivo data – RP 1:1

Table 4.8-4 RP 1:1 Genotoxicity in vivo

Type of test Method/ Guideline	**Species Strain Sex no/group**	**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**

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

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

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

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

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

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

4.8.1.5 In vivo data – RP 3:2

Table 4.8-5 RP3:2 Genotoxicity in vivo

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

±: inconclusive

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

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

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

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

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

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

Parameters	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)**	**Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)**	**Formaldehyde**
Systemic genotoxicity	one test with deficiencies showing some indications for clastogenic effects ; two tests with negative outcome;. limited confirmation that a.s. reached bone marrow	One ambiguous result (cytogenicity bone marrow); one negative result (micronucleus test), limited confirmation that a.s. reached bone marrow	Negative (cytogenetic & micronucleus assay) contradictory results in humans
Local genotoxicity	No data (but see positive in vitro data)	No data (but see positive in vitro data)	Positive (clastogenic in the gastrointestinal tract of rats after oral exposure; clastogenic in the upper respiratory tract of humans after inhalation; DNA-protein cross-links at the site of first contact after inhalation exposure)

4.8.2 Human information

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

4.8.3 Summary and discussion of mutagenicity

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

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

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

4.8.4 Comparison with criteria

Based on the available data and mechanistic considerations of formaldehyde release local genotoxic effects are to be expected from RP 1:1 and RP 3:2. The presently available data for RP 1:1, RP 3:2, FA and Morpholine support the conclusion that germ cells are not affected and according to CLP Regulation 1272/2008/EC, Annex 1, paragraph 3.5.2.1 the germ cell mutagenicity “hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny.” However according to the ECHA CLP guidance 2012, chapter 3.5.1 “genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, "site of contact” genotoxicants)” may be classified as category 2 mutagen in order to provide an indication that the substance could be carcinogenic. Nevertheless, since the substance is already proposed for classification as carcinogenic Cat 1B, there is no need for this further information. Therefore, labeling for mutagenicity according EU Regulation 1272/2008/EC is not required.

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

It is proposed to base classification of RP 1:1 and RP 3:2 on the data of the hydrolysis product formaldehyde. Arguments for and against reading across the carcinogenicity data and C&L conclusion from formaldehyde to RP 1:1 and RP 3:2 are listed in chapter 4.9.4. The same arguments are valid for the read across of mutagenicity category 2. A consistent approach for the read across for these 2 endpoints is necessary.

4.8.5 Conclusions on classification and labelling

Classification for mutagenicity category 2 is required.

4.9 Carcinogenicity

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

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

4.9.2 Human information

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

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

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

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

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

4.9.4 Summary and discussion of carcinogenicity

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

Consequently it is to be expected that the reaction products of 2-hydroxypropylamine and formaldehyde (1:1 and 3:2) show the same local carcinogenic hazard as Formaldehyde.

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

(I) Proposal supported 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 supported by the applicant in the context of the European Biocidal Products Regulation: The formaldehyde releasing substance should not be classified based on the formal consideration as constituent of a product at the time being “supplied to the user”.

Below the arguments for both of the options are summarized:

Table 4.9-2 Arguments for classification of the 1:1 and 3:2 ratio based on “total releasable formaldehyde” or “free formaldehyde” content

supportive arguments for proposal 1: Classification according to releasable Formaldehyde, i.e. Skin Corr. 1, Skin Sens 1, Carc. 1B	**supportive arguments for proposal 2:** Classification according to “free Formaldehyde”, i.e. Skin Corr. 1
Risk through formaldehyde-release in water is covered	Classification usually relates to the substance itself and not to potential release or degradation products which occur during different use scenarios
According to CLP Regulation Annex I, paragraph 1.1.1.3 a WoE evaluation is required for classification and labelling purposes including “information on substances or mixtures related to the substance or mixture being classified”.	
The formaldehyde releaser is difficult to characterise since it shows equilibrium behaviour and having half-lives depending on dilution, temperature and pH.	Analogue to the evaluation of other “substances of concern” or impurities the cut-off values from the GHS system should be considered for the real amount of free formaldehyde
If classification considers the handling, the dilution and the release kinetics should be considered as well: The DT50 of the release was measured as < 1 hour. Each mg RP 1:1 releases 0.28 mg formaldehyde, each RP 3:2 releases 0.45 mg formaldehyde.	Formaldehyde -releasers are designed as transport forms and depot compounds and these benefits of slow continuous formaldehyde release should be considered. Formaldehyde releasers should not be equalized with a pure formalin-solution.
Formaldehyde release is a hydrolysis and occurs with contact with biological tissue and media	
Solutions of formaldehyde releasers only need to be classified if formaldehyde content is above 0.1%	Formaldehyde release is a hydrolysis and occurs in dilutions with water **à** depending on the releaser type this needs dilutions between 1:10 and 1:1000
In vitro genotoxicity data for MBM support the assumption of <u>local</u> genotoxicity and consequent <u>local</u> carcinogenicity	Other examples for substances (oligomers) that contain formaldehyde and are classified according to free formaldeyhde: ● Polyoxymethylen (CAS formaldehyde-polymer = technical plastic) has different properties compared to FA and is classified differently ● Paraformaldehyde itself (degree of polymerization of 8–10 units) is only classified as toxic (T) and corrosive (C) so far Instead of full classification and labelling a warning label could be applied „can release FA with water contact“ A classification of formaldehyde-releasers on the basis of maximal releasable formaldehyde could be considered as an unusual mixture between the classification process and risk assessment which does not justify either of the both procedures

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

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

4.9.5 Comparison with criteria

Genotoxiciy data for the RP 1:1 and RP 3:2 support local genotoxicity, but no systemic genotoxicity. No carcinogenicity studies are available for the RP 1:1 or the RP 3:2. However carcinogenicity data available for the hydrolysis product formaldehyde support classification for category 1B on the basis of human and animal data. Formally “information on substances or mixtures related to the substance or mixture being classified” should be used within a WoE evaluation for classification and labeling. Arguments for classification in Category 1B and arguments from the applicant supporting for non-classification are listed above. Following a WoE evaluation it is proposed to base classification of the RP 1:1 and the RP 3:2 on the data of the hydrolysis product formaldehyde.

4.9.6 Conclusions on classification and labelling

Classification for carcinogenicity, category 1B is proposed.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information – RP 1:1

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

4.10.1.2 Non-human information – RP 3:2

Table 4.10-1 Summary of data for potential fertility effects

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

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

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

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

4.10.1.3 Human information

No human data are available.

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

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

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

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

4.10.2 Developmental toxicity

4.10.2.1 Non-human information – RP 1:1

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

4.10.2.2 Non human information RP 3:2

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

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

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

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

4.10.2.3 Human information

No human data are available.

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

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

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

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

4.10.3 Summary and discussion of reproductive toxicity

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

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

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

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

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4.10.4 Comparison with criteria

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

4.10.5 Conclusions on classification and labelling

No classification for reproductive toxicity is necessary.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity- RP 1:1

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

4.11.1.2 Neurotoxicity – RP 3:2

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

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

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

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

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

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

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4.11.1.4 Immunotoxicity

No data available.

4.11.1.5 Specific investigations: other studies

No data available.

4.11.2 Human information

No data available.

4.11.3 Summary and discussion

Please see summary in 4.11.-1 above..

4.11.4 Comparison with criteria

No relevant neurotoxicological effects are evident at doses below the MTD.

4.11.5 Conclusions on classification and labelling

No classification for STOT SE or RE is necessary.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Preliminary note: The references to key studies are highlighted bold throughout this chapter.

5.1 Degradation

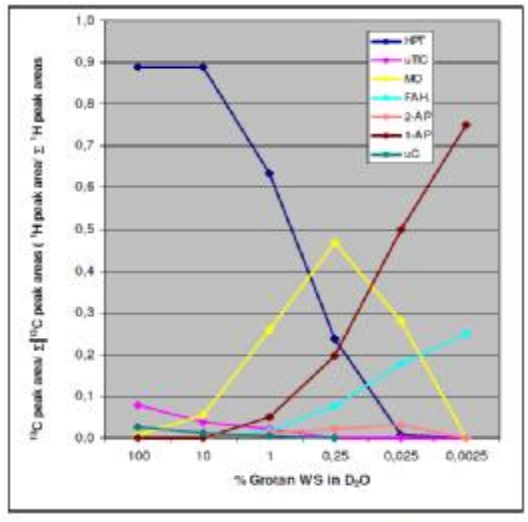
5.1.1 Stability

Hydrolysis

Hydrolysis in water

The hydrolysis of Grotan® WS was studied using ^{1}H and ^{13}C-NMR technique (see **HPT Doc. II-A 7.1.1.1.1, Study A 7.1.1.1.1**). The study is rated Klimisch 2 and was performed without GLP certificate but followed quality assurance standards. Thereby, the dependence of pH, concentration and composition of hydrolysis products has been investigated. Spectra were measured from unbuffered D_2O solutions at 25°C in equilibrium revealing different Grotan® WS concentrations ranging from 0.0025% (v/v) to 100%. The composition of the solutions in D_2O was found to be strongly dependent on the concentration. While at 100 and 10% HPT was the main component, its content decreased with higher dilutions. Formaldehyde, 2-propanolamine 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 completely hydrolysed to formaldehyde hydrate and 2-propanolamine (see Fig. 5.1.1-1).

Fig. 5.1.1-1: Concentrations of the main constituent RP1:1 and the hydrolysis products as a function of the concentration in D2O:



HPT: hexahydro-1,3,5-tris(2-hydroxypropyl)-s-triazine)
uTC: unidentified triazine-like compound
MO: 5-methyloxazolidine
FAH: formaldehyde hydrate
2-AP: 2-aminopropanol(1)
1-AP: 1-aminopropanol(2)
uC: unknown compounds

Compound	Grotan® WS in D_2O					
	100 % (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					
triazine (HPT)	0.8876	0.8883	0.6353	0.2375	0.007	
unknown triazine-like compounds	0.0789	0.0384	0.0215	0	0	
5-methyloxazolidine (MO)	0.0073	0.0557	0.2587	0.4675	0.282	
formaldehyde hydrate (FAH)	0.0000	0.0025	0.0178	0.0750	0.178	0.25
2-aminopropanol(1) (2-AP)	0.0000	0.0034	0.0115	0.0225	0.031	
1-aminopropanol(2) (1-AP)	0.0000	0.0000	0.0495	0.1975	0.503	0.75
unknown compounds	0.0262	0.0117	0.0059	0	0	

In a further test the time-dependent formation of formaldehyde was measured in buffered aqueous solutions containing 1% w/w test materials at different pH values (4, 7 and 9) at 25°C. The highest degree of formaldehyde formation was observed under acidic conditions at pH 4 corresponding also to the highest degree of degradation of Grotan® WS. The lowest amount of formaldehyde was measured at pH 9. It was found that at all pH values the formaldehyde content had reached a plateau after ca. 1 hour.

The pH- dependence of the aqueous hydrolysis of Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1) was investigated using 1% w/w aqueous solutions. This concentration is considered to be higher in comparison to environmentally relevant concentrations. OECD guideline 111 recommends sample concentrations below 10^{-2} M for investigating pH-dependence and hydrolysis under environmentally relevant conditions.

Table 5.1.1-1: Time- and pH-dependent formation of formaldehyde

pH 4		**pH 7 (1.measurement)**		**pH 7 (2.measurement)**		**pH 9**	
time [h]	% H_2CO	time [h]	% H_2CO	time [h]	%H_2CO	time [h]	% H_2CO
0.42	24.82	0.32	18.20	0.37	16.99	0.37	3.03
1.00	26.09	0.93	18.66	0.95	18.66	0.78	3.39
1.78	26.24	1.72	19.72	1.73	18.81	1.57	3.54
2.97	25.88	2.90	19.67	2.92	18.50	2.75	3.59
4.53	25.99	4.47	19.82	4.48	18.86	4.14	3.44
-	-	6.03	19.72	-	-	6.67	3.44

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, Grotan® WS is assumed to be completely hydrolysed to formaldehyde and 1-aminopropanol (= 2-hydroxypropylamine). As the equilibrium was reached rapidly (<1 hour) in the performed test investigating a 1% w/w solution, the hydrolysis half-life DT_{50} is expected to be less than 1 hour under environmentally relevant conditions (temperature, concentration, pH). The study is summarized in the following Table 5.1.1-2.

Table 5.1.1-2 Hydrolysis of Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)

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, 100**	**High degree of hydrolysis at env. relev. concentrations**	**HPT - 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 1h**	
	Conclusion **DT_{50}< 1 h under environmentally relevant conditions**				

Phototransformation in water

There is no study on photolysis of Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1) in aqueous solution available as explained in Doc. III-A 7.1.1.1.2 (Justification for non-submission). The UV spectrum indicates no absorption of light at wave-lengths >290 nm (see Doc III-A 3.4). The US EPA method OPPTS 835.2210 states that the test method is applicable to all chemicals which have a UV-absorption maximum in the range of 290-800 nm. Chemicals with UV absorption maximum of <290

cannot undergo direct photolysis in sunlight. Therefore, the active substance is no candidate for noteworthy photolysis in sunlight and the performance of a test is not necessary. The available information is assumed to be sufficient.

Phototransformation in air

The reaction rate of α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol, the main constituent of Grotan® WS, with OH-radicals in the atmosphere was calculated using AopWin v1.91 (see **Doc. III-A 7.3.1**). The calculated half-life was 46 min corresponding to an OH-radical concentration of $5x10^5$ radicals per cm^3 (cf. Table 5.1.1-3; recommended default value according to EC 2003, part II, chapter 3, 2.3.6.3, p.51).

In the gas phase, α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol is rapidly degraded in air via reaction with OH radicals; degradation by nitrate and ozone is considered to be comparatively negligible. The UV spectrum of the active substance indicates 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 active substance is no candidate for noteworthy direct photolysis in sunlight. Due the low volatility of the main constituent α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol, this degradation pathway is assumed to be of minor importance.

Table 5.1.1-3 Phototransformation in air for the main constituent HPT

Guideline / Test method	Molecule / radical	Rate constant	Molecule/Radical concentration	Half-life (τ1/2)	Reference
Estimation direct photolysis	**h υ**	**0 (expected)**	**-**	**-**	**HPT - Doc. III-A 7.1.1.1.2 Justification for non-submission**
Estimation indirect photolysis (Calculation AopWin v1.91)	**OH**	**$4.98 \cdot 10^{-10}$ cm^3/molecule s**	**$0.5 \cdot 10^6$ / cm^3 (24 h-day)**	**46 min**	**HPT - Doc III-A 7.3.1**
	Ozone	**Negligible compared to reaction with OH radicals**	**-**	**-**	
	NO_3	**Negligible compared to reaction with OH radicals**	**-**	**-**	

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available

5.1.2.2 Screening tests

Ready biodegradability tests

The available biodegradation studies using Contram™ 121 and Grotan® WS as test substance are presented in

Table 5.1.2.2-1.

Table 5.1.2.2-1 Biodegradation of RP 1:1

Guideline / Test method	Test type	Para-meter	Inoculum		Test substance concentr.	Degradation		Reference
			Type	Concen-tration		Incubation period	Degree [%]	
OECD 301D GLP Klimisch 2	**ready**	**BOD**	**Sewage effluent, soil microorg.**	**0.4 mg/L**	**CONTRAM ™ 121 1.75 mg/L**	**28 d**	**30%**	**HPT – Doc III A 7.1.1.2.1/01, Study A 7.1.1.2.1/01**
OECD 301D GLP Klimisch 2	**ready**	**BOD, COD**	**River water**	**0.2 mg/L**	**Grotan® WS 2 mg/L**	**28 d**	**62.7%**	**HPT – Doc III A 7.1.1.2.1/02, Study A 7.1.1.2.1/02**

The biodegradability of RP 1:1 was investigated in 2 studies on ready biodegradability both performed according to OECD Guideline 301D (Closed-Bottle-Test).

In the first study (**HPT – Doc III A 7.1.1.2.1/01)** a mixture of sewage effluent and soil microorganisms was used as inoculum. Degradation of the test substance was calculated on the basis of the COD conducted during this study. The BOD/COD ratio was found to be 29-30% after 28 days. Oxygen consumption was not corrected for nitrification. The toxicity control revealed that at the used test concentration no bacterial toxicity was detected. In this test the pass level for ready biodegradability was not reached.

In the second Closed-Bottle-Test (**HPT – Doc III A 7.1.1.2.1/02)** using river water as inoculum a BOD/COD ratio of 62.7% was calculated. The percentage degradation reached at 21 days 59.3% and increased to 62.7% at day 28. The measured BOD was corrected by the theoretical oxygen consumption due to formation of nitrate and nitrite which were measured simultaneously, while the COD implicated possible partial nitrification. Thus the degradation of the test substance was probably slightly underestimated, although the pass level would have been reached in each case.

However ECHA (2012a) states that the 10-day window does not apply to if the test substance represents a mixture of homologous compounds. Though the RP 1:1 is a mixture the components cannot be considered as homologous in a strict sense. Nevertheless a waiver of the 10 day window is claimed for this case since it is feasible to assume that multi-component substances will lead to a degradation curve characterised by multiphase kinetics with intermediates that have different degradation kinetics and/or that constituents can have sequential degradation.

Also ECHA (2013[4]) states , “The levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10 % of the substance has been degraded; unless the substance is identified as an UVCB …… In this case, and where there is sufficient justification, the 10-day window condition may be waived and the pass level applied at 28 days.”

According to the OECD Guidelines, tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals. As RP 1:1 is an UVCB substance the pass level must be achieved within 28 days. The active substance is considered to be readily biodegradable. This is further supported by the readily biodegradability of the hydrolysis products (see below).

However the two acceptable studies show conflicting results. According to ECHA (2012a) ready biodegradability tests may sometime fail because of the stringent test conditions, in general, and consistent positive test results from test(s) should generally supersede negative test results. It is recommended to consider such differences in stringency and to check the origin of the inoculum in order to check whether or not differences in the adaptation of the inoculum may be the reason (OECD, 2006).

Since both tests were performed according to the same OECD test guideline and under GLP the main difference is the source of the inoculum. No details concerning the adaption of the inoculum of the Daman

[4] http://echa.europa.eu/documents/10162/13562/clp_en.pdf

Ganga River, Vapi in Gujarat, India has been provided by the applicant. According to the annual report 2000-2001[5] of the Central Pollution Control Board of the Ministry of Environment Forest of India the Damanganga river carries the treated/untreated effluents from various industrial estate located in Silvasa, Vapi0.15 and Daman. As per the local fishermen, the fish catch has gone down in recent years. The effluent discharged by CETP Vapi, untreated sewage from Daman and effluents generated by the distilleries in Daman are the major sources of pollution in river Damanganga.

The approximate pollution load received by the river from CETP Vapi in terms of SS, TDS, BOD, COD and NH_3-N is 1.2 T/day, 180.6 T/day, 1.23 T/day, 18 T/day and 6.78 T/day respectively (T = tonnes). Therefore industrial pollution also from untreated waste water is likely. Whether this results in an adaption of the inoculum to triazine compounds/formaldehyde releasing compounds is unclear.

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-hydroxypropylamine (ratio 1:1) (Grotan® WS and CONTRAM™ 121) were performed. According to one study result RP 1:1 is readily biodegradable (62.7% degradation after 28 days).

The interpretation of the biodegradation tests performed with the UVCB substance RP 1:1 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. Therefore also the 10-day window was not applied (cf. ECHA, 2013) since it is feasible to assume that multi-component substances will lead to a degradation curve characterised by multiphase kinetics with intermediates that have different degradation kinetics and/or that constituents can have sequential degradation. ECHA 2013[6] states , "The levels of biodegradation must be achieved within 10 days of the start of degradation which point is taken as the time when 10% of the substance has been degraded; unless the substance is identified as an UVCB. In this case, and where there is sufficient justification, the 10-day window condition may be waived and the pass level applied at 28 days."

Though the two submitted ready tests for RP 1:1 indicate conflicting data the positive results supersede the negative outcome due to the stringency of the method. The main difference of the two studies appeared to be the inoculum. An adaptation to the test compound of the inoculum from the river water could not be conclusively demonstrated. Therefore RP 1:1 is regarded as readily biodegradable.

The equilibrium of hydrolysis is strongly dependent on the concentration in water. At concentration levels being expected in the environment, Grotan® WS is assumed to be completely hydrolysed to formaldehyde and 2-hydroxypropylamine. As the equilibrium was reached rapidly (<1 hour) in the performed hydrolysis test investigating a 1% w/w solution, the hydrolysis half-life DT_{50} is expected to be less than 1 hour under environmentally relevant conditions (temperature, concentration, pH).

In the atmosphere the half-life of α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol, the main constituent of Grotan® WS, was calculated with 46 min (reaction with OH-radicals).

The UVCB substance RP 1:1 is expected to be removed in biological treatment plants as well as in environmental compartments.

[5] http://cpcbenvis.nic.in/ar2001/annual_report2000-01-14.htm

[6] http://echa.europa.eu/documents/10162/13562/clp_en.pdf

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Because of the hydrolysis (**cf. HPT Doc. II-A 7.1.1.1.1**), experimental determination of the distribution coefficient for the reaction product (active substance) and especially for the main constituent HPT is not possible. Therefore, the K_{oc} was estimated according the QSAR model described in EC (2003).

The main component of RP 1:1 is α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol. Therefor the QSARs for soil and sediment sorption for the chemical class for triazines were used according to the TGD, part III (EC, 2003): logKoc = 0.30 logKow + 1.50. Please note that the standard error is 0.38 log unit for this model with n=16. The logKoc is calculated as 1.3 (Koc = 21.8 L/kg).

Further Koc QSAR estimations (Kocwin, v2.00, EPISUITE) are between 0.4 L/kg (log Kow method) and 10 L/kg (MCI method) including fragment correction (cf. HPT Doc III-A 7.1.3).

The range of the QSAR estimations with different models for the Koc is between 0.4 to 21.8 L/kg.

An experimental study for the determination of the adsorption coefficient is not considered necessary based on the fast hydrolysis of RP 1:1.

The low adsorption coefficient indicates high mobility in soils and poor adsorption to sewage sludge and sediment solids

Conclusion:

Adsorption of the reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1) was determined by QSAR estimates. Corrected Koc values for the main component α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol span a range of 0.4 to 21.8 L/kg indicating low adsorption to solid particles in soil and sediment systems.

5.2.2 Volatilisation

Table 5.2.2-1: Vapour pressure

Property	Method	Purity/Specification	Results	**Reference**
Vapour pressure	OECD guideline 104	Contram ™ 121: Purity/Specification: active substance as manufactured (UVCB substance) Batch no.: 24774	$6.4x10^{-5}$ Pa (20°C); $1.3x10^{-4}$ (25°C); 3.9 10^{-3} (50°C) The UVCB substance is unstable; probably hydrolysis products were measured in the gas phase. Test substance was degassed at 80±5°C and ca. 10-5 hPa for 18 hours prior to test.	**Doc. III-A 3; Study A3.2/01**
	EEC A.4	Grotan® WS Purity: UVCB substance (with formaldehyde 26.4-28.0%w/w; 2-hydroxypropylamine 68.0-71.0%w/w) Batch no. 1025145	9.303 $x10^{2}$ Pa (25°C) for the unstable UVCB substance The UVCB substance is unstable; probably hydrolysis products were measured in the gas phase.	**Doc. III-A 3; Study A3.2/02**

Property	Method	Purity/Specification	Results	**Reference**
	Epi Suite 3.12	Purity/Specification: α, α’, α’’-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (main constituent)	4.69 x10^{-7} Pa (Calculation Epi Suite 3.12) The calculation is based on the main constituent, not on the UVCB substance.	**Doc. III-A 3; Study A3.2/03**
Henry´s Law Constant	Calculation based on QSAR	Purity/Specification: α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol (main constituent)	2.55 x10^{-6} Pa xm^3 x mol^{-1} (25°C) (Calculation EPIWIN 3.12) The calculation is based on the main constituent, not on the UVCB substance.	**Doc. III-A 3; Study A3.2/02**

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 the main constituent α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol is 2.55 • 10^{-6} Pa m^3 $mole^{-1}$ indicates that volatilization from aqueous solutions can be assumed to be negligible.

5.2.3 Distribution modelling

No data available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

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 Grotan®WS was determined to be (based on the analyte) -0.48 to -0.61. These values are outside of the domain of the QSAR.

According to ECHA (2012)[7] 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 1:1) 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.

[7] 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

5.3.1.2 Measured bioaccumulation data

There are no experimental data about bioaccumulation available. Because of the hydrolysis properties of RP 1:1 (**cf. HPT - Doc III A7.1.1.1.1**) experimental determination of the BCF is not possible (HPT – Doc III A7.4.2 – Justification).

5.3.2 Summary and discussion of aquatic bioaccumulation

In view of the rapid hydrolysis, a test on aquatic or terrestrial bioconcentration of RP 1:1 seems scientifically not justified. Also the use of a QSAR estimation for aquatic bioconcentration based on a log Kow <1 that is outside the applicability domain is not scientifically sound. The likelihood of bioaccumulation is greatly reduced and the determination of a BCF value is not necessary in this specific case.

A bioaccumulation potential for the main constituent α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol could not be identified based on a very low log Kow value and a DT50 hydrolysis of <1 hour (for RP 1:1).

5.4 Aquatic toxicity

The constituents of the reaction product RP 1:1 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.

Possible pH effects in the environment of the reaction product were not considered, because the STP and receiving compartments are expected to have sufficient buffering.

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

Table 5.4.1.1-1 Acute toxicity to fish

Guideline/ Test method	**Species / Test material**	**Endpoint/ Type of test**	**Exposure**		**Results [mg/L][1]**			**Reference**
			design	**duration**	**LC_0**	**LC_{50}**	**LC_{100}**	
OECD 203 GLP Klimisch 2	***Danio rerio*** Contram™ 121	**Mortality**	**Semistatic**	**96 h**	**50**	**130**	**200**	**HPT - Doc III A7.4.1.1/01**
OECD 203 GLP Klimisch 1	***Oncorhynchus mykiss*** Grotan® WS	**Mortality, sub-lethal effects**	**Semistatic**	**96 h**	**≥ 100**	**-**	**-**	**HPT - Doc III A7.4.1.1/02**

[1]results based on nominal concentrations (measured conc. ≥ 80% of nominal, via formaldehyde and HPA)

CONTRAM™ 121 was tested with the zebra fish *Danio rerio* in a 96 h semistatic test according to OECD Guideline 203 (**HPT - Doc III A7.4.1.1/01, Study A7.4.1.1/01)**. The concentration of the test substance during exposure was monitored indirectly via formaldehyde, resulting in no significant loss of test substance during the test period. Considering nominal concentrations the LC50 was determined to be 130 mg/L.

Grotan® WS was additionally applied in a limit test on the rainbow trout *Oncorhynchus mykiss* (**HPT - Doc III A7.4.1.1/02, Study A7.4.1.1/02)**. At 100 mg/L neither mortality nor behavioural responses or clinical symptoms could be observed within the test period. Analytical measurements of the formaldehyde and 2-hydroxypropylamine content revealed that the deviation from nominal values were <20%.

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 5.4.2.1-1 Acute toxicity to invertebrates

Guideline/ Test method	**Species / Test material**	**Endpoint/ Type of test**	**Exposure**		**Results [mg/L][1]**			**Reference**
			design	**duration**	**EC_0**	**EC_{50}**	**EC_{100}**	
OECD 202/I GLP Klimisch 1	***Daphnia magna*** Contram™ 121	**Mobility**	**static**	**48 h**	**11**	**29**	**75**	**HPT - Doc III A7.4.1.2/01**
OECD 203 GLP Klimisch 3	*Daphnia magna* Grotan® WS	Mobility	static	48 h	0.5	0.72	>1.04	HPT - Doc III A7.4.1.2/02

[1]results based on nominal concentrations

Two tests on acute toxicity to *Daphnia magna* according to OECD Guideline 202 were conducted. The test with CONTRAM™ 121 resulted in a 48 h-EC_{50} value of 29 mg/L (**HPT - Doc III A7.4.1.2/01, Study A7.4.1.2/01)**. Analytical measurements of the free formaldehyde content revealed that the deviation from nominal values were generally <20%.

In a test with Grotan® WS as test substance, an 48h-EC_{50} of 0.72 mg/L was determined for *Daphnia magna* (**HPT - Doc III A7.4.1.2/02, Study A7.4.1.2/02)**. The study obtains clear deficiencies, the results are not plausible compared with other studies and there is no evidence of the actual concentrations tested. Therefore, the test was rated with Klimisch 3.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Studies on chronic fish and invertebrate toxicity using RP 1:1 as test substance were not submitted (cf. **HPT - Doc III A7.4.3.2 – Justification, HPT - Doc III A7.4.3.4 - Justification).**

Please see Table 5.4.2.2-1 for the comparison of the aquatic ecotoxicological profiles of the two UVCB substances RP 1:1 and RP 3:2[8]. From the presented data the two reaction products show comparable toxicity

[8] The UVCB substance "reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio 3:2, short: RP 3:2)" generates predominantly formaldehyde and 2-hydroxyproplamine quite quickly under environmental relevant conditions. The main constituent is N,N'-methylene-bis(5-methyloxazolidine).

despite that the releasable formaldehyde fraction of RP 1:1 is lower (27% to 28%w/w,) compared to RP 3:2. (42 – 49%w/w).

However hydrolysis properties of RP 1:1 and RP 3:2 are similar (cf. **HPT - Doc III A7.1.1.1.1, Doc III A7.1.1.1.1-MBO**). Because both reaction products are produced from the same parent compounds, they will contain the same components, although in a different quantitative composition (for formaldehyde see above). Therefore, no unknown component in "Reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)" is expected which could cause toxic effects on daphnia. Based on these arguments the performance of a chronic test on invertebrates with RP 1:1 as test substance is not expected to give significantly different results than the available test on RP 3:2. Therefore the study on chronic toxicity to *Daphnia magna* with "reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 3:2)" as test substance, which resulted in a NOEC of 1.3 mg/L, is taken for read-across (Table 5.4.2.2-2)

Table 5.4.2.2-1 Comparison of aquatic toxicity data

Endpoint		**RP 1:1**	**RP 3:2**
Acute	Fish	96h-LC_{50} = 130 mg/L (*Danio rerio*)	96h-LC_{50} = 57.7/ 71 mg/L (*Danio rerio*)
	Invertebrates	48h-EC_{50} = 29 mg/L (*Daphnia magna*)	48h-EC_{50} = 28 / 37.9 mg/L (*Daphnia magna*)
	Algae	72h-E_rC_{50} = 6.9 mg/L (*Desmodesmus subspicatus*) 72h-E_rC_{50} = 2.9 mg/L (*Pseudokirchneriella subcapitata*)	72h-E_rC_{50} = 1.8 / 5.7 mg/L (*Desmodesmus subspicatus*)
Chronic	Fish	Not available	Not available
	Invertebrates	21 d-NOEC = 1.3 mg/L (*Daphnia magna*, test substance RP 3:2)	
	Algae	72h-NOE_rC = 0.9 mg/L (*Desmodesmus subspicatus*) 72h-E_rC_{10} = 0.148 mg/L (*Pseudokirchneriella subcapitata*)	72h-NOE_rC = 0.5 / 2.2 mg/L (*Desmodesmus subspicatus*)

Table 5.4.2.2-2 Chronic toxicity to invertebrates of RP 3:2

Guideline / Test method	**Species/ Test item**	**Endpoint / Type of test**	**Exposure**		**Results [mg/L][1]**		**Remarks**	**Reference**
			design	**duration**	**NOEC**	**LOEC**		
OECD 211 GLP, Klimisch 1	***Daphnia magna*** Grotan® Ox	**Re-production**	**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 (biocidal product containing the UVCB substance RP 3:2 as manufactured) according to the OECD Guideline 211 in a semi static system (Doc III A7.4.3.4-MBO, Study A7.4.3.4-MBO). 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 Cl 0-3 mg/L; EC50 26.4 mg/L, Cl 11.6-1608 mg/L; cumulative offspring of survivors). Test item related effects were found for the additional endpoints mobility (NOEC = 8.0 mg/L), intrinsic rate of population growth (NOEC >50 mg/L), and age at first reproduction (NOEC > 20 mg/L). Length and diameter of the parent animals were not affected at

20 mg/L (determined after termination of exposure). However at 20 mg/L mortality of parent animals was 15% and 100% after 4 days at 50 mg/L.

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

5.4.3 Algae and aquatic plants

Table 5.4.3-1 Inhibition on algae of RP 1:1

Guideline /Test method	**Species/Test item**	**Endpoint/ Type of test**	**Exposure** (design, duration)	**Results** [mg/L] **NOE_rC**	**E_bC_{50}**[1]	**E_rC_{50}**[2]	**Remarks**	**Reference**
OECD 201 GLP, Klimisch 2	***Desmodemus subspicatus*** **CONTRAM ™ 121**	**Growth rate**	**static 72 h**	**0.9 (m.c.)** *SD 10, 95%CI 31%*	**3.3 (m.c.)** *SD 19, 95%CI 80%*	**6.9 (m.c.)** *SD 7, 95%CI 29%*	**conc. <80% of nominal (via form-aldehyde)**	**HPT – Doc III A7.4.1.3/1**
OECD 201 GLP, Klimisch 2	***Pseudokirch neriella subcapitata*** **GrotanÒ WS**	**Growth rate**	**static 72 h**	**E_rC_{10}**[3] **0.148 mg/L**	**E_bC_{50}**[3] **0.32 mg/L** *CI 0.16-0.65* mg/L	**E_bC_{50}**[3] **2.95 mg/L** *CI 0.36 – 24.29* mg/L	**conc. <80% of nominal (via form-aldehyde and HPA)**	**HPT - Doc III A7.4.1.3/2**

[1] calculated from the area under the growth curve; [2] calculated from growth rate; [3] corrected for 76% recovery

m.c.: measured concentration,

CONTRAM™ 121 (**HPT - Doc III A7.4.1.3/1, Study A7.4.1.3/1**) was also tested for inhibition of algal growth with the species *Desmodesmus subspicatus*. Analytical monitoring (based on formaldehyde measurement) showed a significant loss of test substance below 20 mg/L (<80 % of the nominal concentrations, mean recovery 96%). Therefore, the effect values were calculated on the basis of measured concentrations. Based on growth rate, a NOEC of 0.91 mg/L and an ErC_{50} of 6.9 mg/L were obtained.

No explanation is given in the study report that addresses the pH deviation of more than one unit. The control showed an increase from pH 7.94 to 9.12. The two lower test concentrations of 2.5 mg/L and 5 mg/L showed an increase from appr. pH 7.5 to pH 9.8. According to OECD (2000)[9] growth of algal test cultures can cause increase of pH due to consumption of HCO_3 ions, though $NaHCO_3$ concentrations have been increased in this test. 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 is not completely maintained for the hydrolysis product 2-hydroxypropylamine with a pK_a of 9.94.

As was shown by Abeliovich and Azov (1976) increased pH ≥8 facilitates penetration into green algae cells (*Scenedesmus obliquus*) of Methylamine (pKa=10.6 according to the SRC PhysProp Database)[10], a related compound to 2-hydroxypropylamine causing disruption of photosynthesis. So pH related effects cannot be excluded for the tested mixture. However pH values decreased after 72 hours in the higher concentrations (10 mg/L, 20 mg/L and 40 mg/L).

[9] http://www.oecd-ilibrary.org/docserver/download/9750231e.pdf?expires=1385738495&id=id&accname=guest&checksum=90E189B53DA5CB93A8280F813D892394

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

Additionally the inhibition of algal growth on the species *Pseudokirchneriella subcapitata* was investigated with Grotan® WS according to a GLP conform study according to OECD 201 (**HPT - Doc III A7.4.1.3/2, Study A7.4.1.3/2**). The analytical control of the test substance concentration showed significant loss of the test substance. The analytical data of a 20 mg/L concentration showed a loss of 76% from the nominal concentration (LOQ for Grotan WS was 10 µg/ml). Corrected for the recovery (93%) the loss was calculated with 82%. Therefore all endpoints were corrected for the 76% recovery. But **Study A7.4.1.3/1** showed that losses increased with lower concentrations therefore the true concentrations might be even lower. Ph-values were in the recommended range, noteworthy is that also the ph-values decreased in the higher concentrations of Study HPT-Doc III A7.4.1.3/01.

A difference of the E_rC_{50} and E_bC_{50} was observed in the above mentioned study. However, differences in descriptors for biomass and growth rate are immanent from a mathematical point of view and quite common. A ratio of 9 is not considered as extreme. One reason is that the selection of the concentrations was not optimal for the endpoint growth rate (resulted in a flat dose-response curve, E_rC50 is outside the tested concentrations). Also a higher growth rate in the test is another contributing factor.

Both studies showed an algicidal effect of RP 1:1 after 24 hours in the highest test concentrations (40 mg/L and 3.2 mg/L).

The NOEC was determined in the study report (Dunett´s test using individual replicate values) with <0.05 mg/L. Based on the flat dose-response curve and the observed difference between biomass integral and growth rate an ErC_{10} of 0.148 mg/L (corrected for 76% recovery) is suggested.

Ratte (1998) showed that a longer test duration, high growth rate, and flat dose-response relationship are expected to evoke large differences between the E_bC_{50} and E_rC_{50}. According to additional theoretical considerations of Nyholm (1985, quoted in Ratte, 1998), the EC_{10} is expected to be less dependent on the endpoint selected. This is another argument for the use of the EC_{10}.

The studies on the toxicity towards algae demonstrate that the reaction product of para-formaldehyde and 2-hydroxy-propylamine (ratio 1:1) 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) of RP 1:1

Guideline / Test method	Inoculum / Test item	Endpoint / Type of test	Exposure		Results[1]			Remarks	Reference
			design	duration	EC_0	EC_{50}	EC_{80}		
OECD 209 GLP Klimisch 2	**Activated sludge, municipal** CONTRA M™ 121	**Inhibition of respiration**	**static**	**3 h**	**23 mg/L**	**110 mg/L**	**560 mg/L**	**nominal conc.**	**HPT - Doc III A7.4.1.4/1**
OECD 209 GLP Klimisch 1	**Activated sludge, industrial** **GrotanÒ WS**	**Inhibition of respiration**	**static**	**3 h**		**29 mg/L** (CI 25 – 33 mg/L)		**nominal conc.**	**HPT - Doc III A7.4.1.4/2**

[1].: nominal concentration;

The acute toxicity of the active substance "reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)" towards bacteria was tested according to OECD Guideline 209 in a GLP conform study by determining the inhibition of respiration in sludge samples from biological treatment

plants receiving predominantly domestic sewage (cf. Table 5.4.4-1). In the test, the 3 h-EC_{50} was established at a concentration of 110 mg/L and the EC_0 at 23 mg/L (cf. **HPT - Doc III A7.4.1.4/1, Study A7.4.1.4/1)**. Not method for the calculation of these endpoints is described in detail in the study report. Please note that no NOEC or EC10 was provided in the study. The EC20 is outside the tested concentration range starting with 62.5 mg/L (lowest concentration of 32 mg/L could not be used due to experimental problems).

In a second study using sludge from an industrial treatment plant a 3 h-EC50 of 29 mg/L was obtained cf. **HPT - Doc III A7.4.1.4/2, Study A7.4.1.4/2)**. The result is lower than the other reported value by a factor of 3.8 but because the variability of the method OECD 2009 suggests that in many cases it is sufficient to express the results additionally in order of magnitude.

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

CLP:

Aquatic Acute 1:

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

Lowest L(E)C_{50} value: E_rC_{50} (algae) = 2.9 mg/L

- **No classification**

Studies used:

- Doc. III-A 7.4.1.1/01: Institut Fresenius, Study on the Acute Toxicity towards Fish of "Contram 121" according to OECD-Test Guideline 203 -> **LC_{50} (fish) =130 mg/L**
- Doc. III-A 7.4.1.2/01: Institut Fresenius, OECD 202, Part I Study on the Acute Toxicity towards Daphnia of "Contram 121" -> **EC_{50} (crustacea) =29 mg/L**
- Doc. III-A 7.4.1.3/01: Institut Fresenius, OECD 201, Study on the Toxicity towards Algae of "Contram 121" according to OECD-Test Guideline 201 -> **E_rC_{50} (algae) =6.9 mg/L**
- Doc. III-A 7.4.1.3/02: Jai Research Foundation, OECD Guideline 201, Alga (*Selenastrum capricornutum*) Growth Inhibition Test with Grotan WS -> **E_rC_{50} (algae) =2.9 mg/L**

Aquatic Chronic Categories:

The reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1) is rapidly degradable, adequate chronic toxicity data are available for algae and cladocerans (read-across from RP 3:2). The algae E_rC_{10} is 0.148 mg/L, which leads to a classification with Aquatic Chronic 3.

The lowest long-term effect E_rC_{10} value of 0.148 mg/L was derived from the green algae study (*Pseudokirchneriella subcapitata*) with RP 1:1 (test item Grotan® WS). The reaction product is readily biodegradable and has a DT50 hydrolysis of <1 hour. Therefore a classification as Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects according to the 2nd ATP of Regulation (EC) No 1272/2008 is proposed.

The result from the algae study was used for classification despite some deficiencies. The endpoints E_rC_{50} and E_bC_{50} differ by a factor of 9. One reason is that the selection of the concentrations was not optimal for the endpoint growth rate (resulted in a flat dose-response curve, calculated E_rC_{50} is outside the tested concentrations). Also the confidence interval for the E_rC_{50} was with 0.36 – 24.29 mg/L very high.
The NOEC was determined in the study report (Dunett´s test using individual replicate values) with <0.05 mg/L. Based on the flat dose-response curve and the observed difference between biomass integral and growth rate an E_rC_{10} of 0.148 mg/L (corrected for 76% recovery) is suggested.
Ratte (1998) showed that a longer test duration, high growth rate, and flat dose-response relationship are expected to evoke large differences between the E_bC_{50} and E_rC_{50}. According to additional theoretical considerations of Nyholm (1985, quoted in Ratte, 1998), the EC_{10} is expected to be less dependent on the endpoint selected. This is another argument for the use of the EC_{10}.
The algae 72h-NOErC for RP 1:1 (test item Contram™ 121) from the second green algae study with *Desmodesmus subspicatus* is 0.9 mg/L, which supports the proposed classification. However this study has also some deficiencies concerning the variability of the pH value in the control and in some test concentrations.
Nevertheless algae have been shown to be the most sensitive species in aquatic acute toxicity tests. The chronic Daphnia study according to OECD Guideline 211 with RP 3:2 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 >100 mg/L are available, which in combination with a log K_{ow} <1 would not lead to a classification.

Aquatic Chronic 1:

- **è No classification**

Aquatic Chronic 2:

- **è No classification**

Aquatic Chronic 3:

- **è Classification with Aquatic Chronic 3**

Studies used:

- Doc. III-A 7.1.1.2.1/02: Jai Research Foundation, OECD 301D, Ready Biodegradability of Grotan WS -> **62.7% degradation at day 28**, waiver of the 10-d window claimed based on the UVCB characteristics of the test item (multi-component substance)
- Doc. III-A 7.1.1.1.1**:** Fraunhofer ITEM, Hydrolysis of the equilibrium mixture of hexahydro-1,3,5-tris(2.hydroxypropyl)-s-triazine and N,N-methylene-bis-(5-methyloxazolidine), 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} = -0.48 – 0.61**

- Doc. III-A 7.4.1.1/01: Institut Fresenius, Study on the Acute Toxicity towards Fish of "Contram 121" according to OECD-Test Guideline 203 -> **LC_{50} (fish) =130 mg/L**
- Doc. III-A 7.4.3.4: SGS Institut Fresenius, OECD-Guideline No. 211 (*Daphnia magna* Reproduction Test), Study on the Chronic Toxicity towards Daphnia of „Reaction Product of Paraformaldehyde with 2-Hydroxypropylamin (Relation 3:2)" -> **NOEC (crustacea) =1.3 mg/L**
- Doc. III-A 7.4.1.3/01: Institut Fresenius, OECD 201, Study on the Toxicity towards Algae of "Contram 121" according to OECD-Test Guideline 201 -> **NOE_rC (algae) =0.9 mg/L**
- Doc. III-A 7.4.1.3/02: Jai Research Foundation, OECD Guideline 201, Alga (*Selenastrum capricornutum*) Growth Inhibition Test with Grotan WS. -> **E_rC_{10} (algae) =0.148 mg/L**

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

CLP:

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

Classification and Labelling			**Justification**
GHS Pictograms		-	No classification for acute toxicity is proposed since for all three tropic levels $L(E)C_{50}$ values >1mg/L are available. Chronic Toxicity: Rapidly degradable substance for which adequate chronic toxicity data are available for daphnia and algae. Lowest chronic value is the E_rC_{10} from algae with 0.148 mg/L -> Aquatic Chronic 3.
Signal words		-	
Classification		Aquatic Chronic 3	
Hazard statements		H412: Harmful to aquatic life with long lasting effects	
Precautionary Statements	**General**	-	
	Prevention	P273: Avoid release to the environment	
	Response	-	
	Storage	-	
	Disposal	P501: Dispose of contents/container in accordance with local/regional/national/ international regulations (to be specified).	

6 OTHER INFORMATION

Not available

7 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	Contram 121 - Method of manufacture of the active substance, Lubrizol Hamburg Lubrizol Deutschland GmbH, M. Gierschmann, M. P. Scholz, 15.11.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.7/01	2007a	Purchased material specifications sheet, Product: Contram 121/BC6121. Lubrizol Deutschland GmbH, 16.11.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.7/02	2007a	Release specification of Grotan WS. Schülke & Mayr GmbH, S. Hendrich, 20.11.2007 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.7/03	2007b	Determination of the Formaldehyde content of different batches CONTRAMTM 121: 1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol [1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol, α,α',α"-trimethyl-] N,N’,N’’-Tris(beta-hydroxypropyl)hexahydro-1,3,5-triazin, (CAS# 25254-50-6) Quality Control Laboratory – Lubrizol Deutschland GmbH, Document No. 57, 17.12.2007 GLP not applicable, unpublished	Y	Lubrizol
A2.7/04	2007b	Formaldehyde content of different batches of Grotan WS Schülke & Mayr GmbH, S. Hendrich, 20.11.2006 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.7/05	2007c	Chargenvergleich von verschiedenen Mustern - α,α',α"-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol Spectral Service GmbH, Analysenbericht SMN18728E, 21.2.07 non GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A2.7/06	2008	Hydrolysis of the equilibrium mixture of hexahydro-1,3,5-tris(2.hydroxypropyl)-s-triazine and N,N-methylene-bis-(5-	Y	Schülke & Mayr +

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		methyloxazolidine) Fraunhofer ITEM (Dr A Preiss), Report 25.2.08 non GLP, unpublished		Lubrizol
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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 “α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol” (HPT) for life-cycle step production at Schülke & Mayr GmbH. S. Hahn, J. Regelmann, Fraunhofer Institute of Toxicology and Experimental Medicine, Department Chemical Risk Assessment, 24.7.2007 GLP not applicable, unpublished	Y	Schülke & Mayr
A2.10/02	2007	Determination of total aldehyde in the waste water stream of Schülke & Mayr GmbH. Schülke & Mayr GmbH, Dr. Susanne Hendrich, 2.7.2007 (unpublished) non GLP, unpublished	Y	Schülke & Mayr
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A3.1.3/03	2007	Determination of the Density of CONTRAM™ 121. Lubrizol Deutschland GmbH, Hamburg 17.12.2007 No GLP, unpublished	Y (Exist.)	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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A3.4/02	2007	UV/VIS Scan of Grotan WS Schülke & Mayr Analytical Service, 18.6.2007 No GLP, unpublished	Y	Schülke & Mayr
A3.4/03	2007	Determination of the Infrared (IR) Spectrum of CONTRAMTM 121. Lubrizol Industrial Additives, Hamburg July 4, 2007 No GLP, unpublished	Y	Lubrizol
A3.4/04	2007	IR-Spectrum of Grotan WS Analytical Laboratory Schülke & Mayr, Dr. S. Hendrich, 14.12.2007 No GLP, unpublished	Y	Schülke & Mayr
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A3.4/07	2007	Mass spectrum of Contram 121 Weber, L. University of Bielefeld, 09.07.2007 No GLP, unpublished	Y	Lubrizol
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A3.2/01	2002	Contram 121, Batch No. 24774, Vapour Pressure. Siemens Axiva Labor Sicherheitstechnik, Rep. No. 20011542.01, Feb. 13, 2002 GLP, unpublished	Y	Lubrizol
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A3.11/01	2000	Flammability of Grotan WS. Jai Research Foundation, Study No. 2693, Jul. 28, 2000 GLP, unpublished	Y	Schülke & Mayr
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A3.14	2007b	Physical and chemical data of Grotan WS, Schülke & Mayr, Research and Development Jan. 10, 2007 No GLP, unpublished	Y	Schülke & Mayr
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A3.17	2007	Reactivity towards container material: CONTRAM™ 121. Michael P. Scholz, Lubrizol, 19.07.2007 GLP not applicable, unpublished	Y	Schülke & Mayr + Lubrizol
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Chapter 5	ECHA	2012a	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, 2014-03-14	N	-
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8 ANNEXES

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