Committee for Risk Assessment

RAC

Annex 1

Background document

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

Reaction products of paraformaldehyde with 2-hydroxypropylamine (ratio 1:1); [HPT]

EC Number: -
CAS Number: -

CLH-O-0000001412-86-89/F

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

Adopted

4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

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

Version number: 2 **Date: 12 December 2014**

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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

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

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	

RAC general comment

The biocidal active substance "*reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)*" is a UVCB substance prepared by the reaction of paraformaldehyde and 2-hydroxypropylamine. The active substance originally notified as α,α',α"-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol, shortened to HPT, according to the biocidal products Directive 98/8/EC, was renamed Reaction products of paraformaldehyde with 2-hydroxypropylamine (referred to throughout this document as RP 1:1).

UVCB substances are identified by their source and manufacturing process. In addition, the active substance is specified by the main identifier "*content of releasable formaldehyde*" which is typically 28% (range of 26 – 30% w/w). The active substance (RP 1:1) is applied in aqueous solutions where, depending on the environmental conditions, it hydrolyses completely to formaldehyde and 2-hydroxypropylamine. Also the active substance is expected to hydrolyse completely once the substance has entered the human or animal body.

The active substance and the biocidal products are handled and marketed as aqueous solutions which contain no organic solvents.

While this opinion covers RP 1:1, another closely related UVCB called "Reaction product of paraformaldehyde and 2-hydroxypropylamine (RP 3:2) is also produced and is of relevance to this evaluation by RAC

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

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

In this opinion, RAC documents the weight of evidence on the intrinsic properties of RP 1:1 in the order of data on RP 1:1 (the UVCB substance to be classified), data on RP 3:2 and finally, data on the hydrolysis products formaldehyde and and 2-

hydroxypropylamine.

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

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**

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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.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The DS included data on both reaction products of paraformaldehyde with 2-hydroxypropylamine (RP 1:1) and (RP 3:2). For RP 1:1 there is one oral rat study and two dermal rat studies. The LD_{50}'s were 960 mg/kg bw in the oral and above 2000 mg/kg bw for dermal. For RP 3:2 there were three oral rat studies and three dermal rat studies. The LD_{50}'s were 630 mg/kg bw for acute oral toxicity and 790 mg/kg for acute dermal toxicity. No data was available on inhalation toxicity.

Although the data could be considered to support classification, the DS stated that the effects were due to the corrosivity of the substance and therefore proposed no classification for acute toxicity.

Comments received during public consultation

Three Member State Competent Authorities (MSCA) suggested that classification for acute toxicity may be applied. Two MSCAs proposed that in addition to classification for skin corrosivity, RP 1:1 should be classified as: Acute Tox. 4 (oral); H302, Acute Tox. 3 (dermal); H311, Acute Tox. 4 (inhalation); H332.
Two MSCAs also proposed addition of the supplemental hazard information statement EUH071 (Corrosive to the respiratory tract) and one MSCA raised the possibility of adding EUH029 (Contact with water liberates toxic gas).

Assessment and comparison with the classification criteria

Acute oral toxicity

<u>RP 1:1</u>
An LD_{50} of 960 mg/kg bw was estimated in the acute oral toxicity study on RP 1:1 according to OECD TG 401 (Schülke & Mayr, 2000). Macroscopic findings such as 'congestion of stomach, intestine and lungs, mottling in liver' are mentioned in table 4.2-3 of the CLH report. Information on the severity and the dose-response of these effects is lacking, and whether the stomach lesions were likely to have contributed to the mortality remains unclear. Additional information in the document CLH-Rep_ATT_HPT Doc III A (further on cited as "RP 1:1 Doc III A") states that 'varying degree of mucosal congestion/erosion in the glandular part of the stomach and congestion/mucus exudation in small intestine, also emphysema/congestion of lungs and mottling in liver' were observed. In general, congestion is related to the premortal circulatory failure and is expected as a nonspecific premortal finding. Erosive lesions, if present due to the irritative properties of the substance (which is not known, as no microscopy has been done), should start in the forestomach and should also be most prominent in this region. Local erosions alone – unlike ulcerations – depending on the severity and progression of the lesion (in general) are unlikely to be lethal (and then only if of high severity after prolonged duration of exposure, illness delayed mortality may not be excluded). Other effects were reported in Doc III A6.1.1 as lethargy, abdominal breathing, gasping and piloerection at the day of dosing in all treatment groups. A slight decrease in body weight gain of survivors were seen.

Concerning the DS proposal not to classify for acute oral toxicity due to the classification as a corrosive substance, RAC does not find a general disclaimer on acute toxicity for non-classification of corrosive substances in the CLP Regulation. In addition, there are no data that clearly indicate that the mechanism of toxicity was corrosivity. No details on the macroscopic findings were given. Congestion may be concluded from reddening of the

stomach mucosa, but congestion alone is not predictive of a corrosive/ulcerative event. How the finding 'erosion' was characterised, remains unclear, in particular as the macroscopic finding reported as 'congestion/erosion' in the glandular stomach was not verified by microscopy.

The lack of evidence for RP 3:2 to cause corrosive (ulcerative) effects in the forestomach region supports the interpretation that the mortalities of RP 1:1 are systemically induced.

RP 3:2
The oral LD_{50} of 630 mg/kg bw was derived from a study in accordance with OECD test guideline (TG) 423 where mortality at 2000 mg/kg bw was 100% and no effects were seen at 200 mg/kg bw (Bode Chemie, 2002). Local effects along the upper GI tract that could indicate corrosivity of the 10% test solution in corn oil were not reported for this study. Also two further oral studies (Schülke & May, 1977, 1979) did not show local effects at test concentrations of 8% and 10%, respectively, in aqueous 0.9% NaCl solution. The DS explicitely noted that surprisingly no local effects were detected in the oral studies. In both studies, the clinical effects in surviving rats were reversible within 24 hours.

In conclusion, an OECD TG 423 study on RP 3:2 revealed a LD_{50} value of 630 mg/kg bw and two other studies with a test design similar to OECD TG 401 resulted in oral LD_{50} values of 750 and 900 mg/kg bw. Thus, RAC concluded that the data warrants to classify RP 3:2 (based on the studies on RP 3:2) as Acute Tox. 4; H302 (Harmful if swallowed) according to CLP (oral LD_{50} guidance values for this category from 300 to 2000 mg/kg bw).

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

2-Hydroxypropylamine
In the document CLH-REP_ATT_Appendix HPA_DV018252-32 (referred to as "Doc Appendix HPA" throughout this document) two studies were cited revealing an LD_{50} of 4260 mg/kg bw (Smyth *et al.*, 1949) and an LD_{50} of 2100 mg/kg bw (Carreon & Yakel, 1981).

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

RAC agrees to classify RP 1:1 (based on the oral study on RP 1:1 and consistent to RP 3:2) as **Acute Tox. 4; H302 (Harmful if swallowed)** according to CLP Regulation (oral LD_{50} guidance values for this category from 300 to 2000 mg/kg bw).

Acute dermal toxicity

RP 3:2
The lowest LD_{50} of 760 mg/kg bw was estimated for female rats (males and females combined LD_{50} 790 mg/kg bw) (Bode Chemie, 2002). No local skin effects were observed in all animals at this dose. At 2000 mg/kg bw (test substance was undiluted at this dose), 1/5 males had erythema and slight oedema (5/5 males died on day 1-7), 4/5 females had slight to severe erythema and slight to severe oedema (5/5 females died on day 1-7). As no indication on skin necrosis and scab formation was reported in only 2/5 female animals at 2000 mg/kg bw and 4/5 males died without any skin effects, the mortalities observed can not be explained by corrosive effects.

RAC proposes that based on the lowest acute dermal LD_{50} value of 760 mg/kg bw in

female rats, RP 3:2 should be classified as Acute Tox. 3; H311 (Toxic in contact with skin) according to CLP (dermal LD_{50} guidance values for this category from 200 to 1000 mg/kg bw). As mortalities occurred without local skin effects or scab formation due to necrotic precursor lesions were seen in the surviving animals at the end of the 14 day observation time, skin lesions are unlikely to be the cause of mortalities.

Formaldehyde
Formaldehyde is classified in CLP, Annex VI as acute dermal toxicity, category 3; H311 (Toxic in contact with skin).

2-Hydroxypropylamine
In Doc Appendix HPA the study of Smyth et al. (1949) calculated an LD_{50} of 1640 mg/kg bw and the study of Carreon & Yakel (1981) identified an LD_{50} of 1850 mg/kg bw, both in rabbits.
There is no entry in CLP, Annex VI for acute dermal toxicity. Some self-classifications as Acute Tox. 4; H312 (Harmful in contact with skin) are available.

RP 1:1
Two dermal acute studies on RP 1:1 revealed LD_{50} values > 2000 mg/kg bw when undiluted test substance was dermally applied. The study of Becker Chemie (2002), conducted according OECD TG 402, is taken as the most informative study. No deaths were observed at 2000 mg/kg bw in a preliminary test on two female rats. One out of 5 females was found dead at this dose level on day 4 in the main (limit dose) study with clinical signs of reduced activity, abdominal position, paleness, piloerection as well as reduced body and abdominal tone. No effects were seen in other rats. Black colouration was reported as local effects in this female.

Skin reaction (slight to well defined erythema and yellowish disclouration after patch removal, in 3 rats additional hardening with dark discolouration) were reported (assumed by the RAC to have been observed at the end of the 24 h exposure time). Erythema was still present up to 24 h after patch removal. Over the following days discolouration, hardening and desquamation was observed which was not fully reversible up to day 14.

No mortality and no other effect (bw, clinical signs) were recorded in the OECD TG 402 study of Schülke & Mayr (2002). Skin reactions were not recorded.

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

RAC agrees with the view of three MSCA that corrosivity does not cover the acute toxicity classification. RAC noted that a read across to RP 3:2 and to formaldehyde would support a classification as Acute Tox 3; H311. However based on the available studies for RP 1:1 it appears that the potential of dermal toxicity differs from RP 3:2 and formaldehyde and RAC gives more weight to the studies on RP 1:1. RAC concludes that **classification of RP 1:1 for dermal acute toxicity is not warranted**.

Acute inhalation toxicity

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

Formaldehyde
There are acute inhalation studies (see Formaldehyde Core Document) suggesting that corrosive effects in the upper respiratory effects may contribute (possibly in addition to other effects) to lethality: histopathological examination revealed excessive mucus

secretion, mucociliary dysfunction, single cell necrosis, and discontinuous nasal epithelium with erythrocyte leakage following 4 h of exposure of rats to formaldehyde gas concentrations of 12 µg/L (Bhalla *et al.*, 1991). Higher concentrations (0.6-1.7 mg/L) resulted in haemorrhage and oedema of the lung as well as oedema in liver and kidneys and hepatocyte necrosis (Skog, 1950). The Formaldehyde Core Document indicates a LC_{50} of 0.6 mg/L (4 h).
Formaldehyde is classified in CLP, Annex VI as acute inhalation toxicity, category 3; H331 (Toxic if inhaled).

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

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

The CLP Guidance (version 4.1, 2015), 3.1.2.3.2 states that 'Corrosive substances (and mixtures) may be acutely toxic after inhalation to a varying degree and by different modes of action. Therefore, it is not possible to estimate the acute inhalation toxicity from the corrosivity data alone.

The DS considered acute inhalation toxicity, category 4 (H332) for RP 1:1 based on the read across from formaldehyde vapour to released mist with 28% formaldehyde content, but found the classification for acute (inhalation) toxicity redundant for corrosive substances.

RAC considers read across to formaldehyde justified as RP 1:1 contains 28% releasable formaldehyde and agrees on Acute Tox. 4 (as suggested by two out of three MSCA supporting classification for acute inhalation toxicity) based on the formaldehyde classification (Cat. 3) and taking the maximum amount of releasable formaldehyde into account.

Acute Tox. 4 is considered justified assuming that the acute inhalation toxicity of RP 1:1 is totally dependent on 28% releasable formaldehyde. For RP 1:1 the LC_{50} of about 1.8 mg/L (factor of 3 applied on a LC_{50} of 0.6 mg/L (4h) for formaldehyde) for RP 1:1 would result. For mists, this is equivalent to Cat. 4. RAC thus agrees to classify RP 1:1 as **Acute Tox. 4; H332 (Harmful if inhaled)**

This is consistent with the observation that acute toxicity values for the oral and dermal route demonstrated lower potency of RP 1:1 than formaldehyde to cause acute toxic effects. RAC discussed uncertainties that remain with regards to the actual emitted concentrations in air (in the gaseous phase or aqueous solution) as hydrolysis data in contact with biological tissues are lacking, and uncertainties that may result from nonstable intermediates which could also contribute to the acute inhalation toxicity.

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

RAC notes that the CLP criteria on EUH071 are not clearly defined. EUH071 can also be

applied to inhaled corrosive substances not tested for acute inhalation toxicity. According to CLP, Annex II, ,1.2.6 (which states *'For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled.'*) EUH071 may then be appropriate whithout a corresponding classification for acute inhalation toxicity.

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

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

RAC discussed that the liberation of toxic gas after contact with water will not be the main concern as sufficiently high amounts of toxic gase may not immediately be produced. Formaldehyde will also be generated and released without contact with water as aqueous conditions are given under normal room air conditions in contact with mucous membranes (of the eye, the respiratory tract and the upper GI tract) and in contact with sweaty skin. It is also of note that the CLP, Annex 11, 1.2.1 foresees the additional labelling with EUH029 only for substances classified for acute toxicity in category 1, 2 or 3 and not for Acute Tox. 4 substances.

RAC agrees that EUH029 is not warranted.

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

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

Therefore no classification is required.

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

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

One MSCA remarked that the classification STOT SE is not covered by the classification for skin corrosivity. With regards to STOT SE, this MSCA agreed that no classification is required.

Assessment and comparison with the classification criteria

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

Formaldehyde
For formaldehyde, there is no entry in Annex VI on STOT SE; some notifiers self-classified for STOT SE (1 or 3).

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

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

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

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

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

RAC agrees with the DS that **no classification on STOT SE is warranted**, and that the potential for respiratory tract irritation is covered by the classification of RP 1:1 as corrosive.

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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

Comments in agreement with proposed classification for Skin Corrosivity Cat. 1 without subcategorisation were submitted by three MSCA.

Assessment and comparison with the classification criteria

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

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

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

RP 1:1
Irritant and corrosive properties were observed for RP 1:1 in two studies conducted according to OECD TG 404 (Becker Chemie, 2002, Schülke & Mayr, 2000). Exposure periods of 3 min and 1 h were also tested in the study of Becker Chemie (2002) and revealed only well defined erythema at 4 h post-exposure. Some evidence for damage of deeper skin layers such as induration, discolouration, scab formation and desquamation was noted after 4 h exposure; the effects were not reversible.
The study of Schülke & Mayr (2000) resulted after 4 hours exposure in destruction of skin tissue (eschar formation) that was observed at day 7 in 2 rabbits. The effects were reversible on Day 14. Additional information on the exposure duration originated from Doc HPT Doc III A.

The DS relied on the translation rules that suggested to translate corrosive substance (R34) to Skin Corr. Cat. 1B and to read across to formaldehyde, also classified in Cat. 1B.

As RP 1:1 was tested for skin irritation/corrosion in undiluted form which should not contain relevant concentrations of formaldehyde or 2-hydroxypropylamine, the observation of corrosivity supports either that RP 1:1 itself has corrosive properties or that a sufficiently rate of hydrolysis will occur within a short period of exposure (within 4 h) that caused corrosive effects by the hydrolysis products (formaldehyde and 2-hydroxlpropylamine).

Based on the available studies on RP 1:1, RAC proposes classification for skin corrosivity. RAC gives more weight on the available studies on RP 1:1 than only on the read across and general recommendations from the translation period. Based on the observation that

exposure durations up to 1 h did not induce corrosive effects and 4 h did (Becker Chemie, 2002), RAC agrees on the **classification as Skin Corrosive 1C; H314 – Causes severe skin burns and eye damage** (according to Table 3.2.1, CLP Guidance).

4.4.2 Eye irritation

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

4.4.2.1 Non-human information for RP 1:1

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

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

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

4.4.2.2 Non-human information for RP 3:2

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

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

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

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

No human data available.

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

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

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

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

4.4.2.5 Summary and discussion of eye irritation

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

4.4.2.6 Comparison with criteria

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

4.4.2.7 Conclusions on classification and labelling

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

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

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

Comments received during public consultation

It is pointed out by one MSCA although no labelling is required as the substance is also skin corrosive the substance should be classified as Eye Dam. 1.

Assessment and comparison with the classification criteria

RP 3:2
RAC recommends to classify for Eye Dam. 1 as for corrosive substances the risk for severe eye damage is implicit and has been demonstrated in animal studies. A separate labelling with H318 is not needed.

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

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

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

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

2-Hydroxypropylamine
Studies reporting corrosive properties to eyes were documented in Doc Appendix HPA.
There is no Annex VI entry on a separate classification for eye irritation/damage on 2-hydroxypropylamine, but the majority of notifiers classify the substance as Eye Dam. 1.

RP 1:1
The eye irritation study of Schülke & Mayr (2000) according to OECD TG 405 revealed non-reversible cornea lesions of RP 1:1 that support the classification as Eye Dam. 1.

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

CLP guidance (version 4.1, 2015) stipulates in section 3.3.2.4:

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

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

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

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

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

In previous cases of corrosive substances, RAC decided not to propose a separate classification on serious eye damage. For RP 1:1, RAC agrees to classify as Eye Dam. 1. Although for corrosive substances the risk for severe eye damage is implicit (and testing should be avoided), in this case severe eye damage has been demonstrated in an animal study on RP 1:1 and **justifies a separate classification as Eye Dam. 1. Separate labelling with H 318 is not needed**.

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.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

Comments in agreement with skin sensitisation 1A by two MSCA.

Assessment and comparison with the classification criteria

RP 3:2
A total of 3.1% or 55 patients out of 1786 patients showed positive reaction to RP 3:2 (Geier et al., 1997). A more recent study (DeGroot et al., 2010) reviewed five patch test studies on patients who were metalworkers with suspected contact dermatitis and who had contact with metal working fluids containing RP 3:2. Positive reactions were found in 2.3 to 6.7% of metal workers (see Table 2 in this publication). These relatively high frequency meets the criteria (selected workers with known exposure or dermatitis is ≥1.0%) for a subcategorization a as skin sensitisation Cat. 1A.
In addition animal data support subcategory 1A (≥60% responding at >0.1% to ≤1% intradermal induction dose based on results from the study of Anderson et al., 1984).

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

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

RP 1:1
Human data on RP 1:1
No information available.

Animal data on RP 1:1

A GPMT study according to OECD TG 406 (with some contradictory observations related to the irritative effects in the preliminary and main studies) (Lubirzol Corporation, 2001) is available supporting skin sensitization Cat. 1A due to 90% positive response to 1% induction concentration.

Another GPMT study was considered as of limited validity for several reasons. Intradermal injection of 1% test substance in distilled water resulted in discrete or pathy erythema, no local effects was observed after topical application of undiluted test material (while strong irritation was expected). A 40% positive response at 24 h was observed with an intradermal induction dose of 1%, which would support a classification for Skin Sens. Cat. 1B.

Based on the evidence from the GPMT study of Lubrizol Corporation (2001) (criteria ≥60% responding at >0.1% to ≤1% induction dose) and on the supporting human/animal evidence from read across to RP 3:2 and formaldehyde, RAC agrees with the proposal by the DS to classify RP 1:1 for skin sensitisation, as **Skin Sens. 1A; H317 (May cause an allergic skin reaction).**

4.6.2 Respiratory sensitisation

No data are available.

4.7 Repeated dose toxicity

4.7.1 Non-human information RP 1:1

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

Route	duration of study; guideline	Species Strain Sex no/group	dose levels frequency of application	identity as given in study report	Results / Remarks	LO(A)EL	NO(A)EL	Reference
Oral (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:	250 mg/kg bw	100 mg/kg bw	Schülke & Mayr (2002); DocIIIA6.3.1/02

					reduced kidney weight. Dose-range finding for 90d study			
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 6 % in peanut oil; once daily, 7days per week	Contram 121 batch 24774	≥ 80 mg/kg bw: clinical signs (breathing sounds), mortality, lesions of larynx and pharynx; 150 mg/kg bw: lesions of oesophagus in f	80 mg/kg bw	30 mg/kg bw	**Lubrizol Deutschland GmbH (2002); DocIIIA6.4.1/01**
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	0, 100, 300, 900 mg/kg bw = 0, 2, 6, 18% in corn oil;	Contram MBO FA	High dose: high mortality (termination day 6); mid	100 mg/kg bw/day	-	Bode Chemie (2002); DocIIIA6.3.1/02

		5 m & 5 f	once daily, 7 d/ week	42.28%	dose: local effects in the stomach, mortality; low dose: body weight and food consumption↓; dose range finding study			
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.

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

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

Oral route

RP 3:2
The most relevant study is a 90-day study (Bode Chemie, 2002, in accordance with OECD TG 408, version 1998) on 10 male and 10 female rats which resulted in mortalities of 3 males (day 49-75) and 5 females (day 49-79) that received 180 mg/kg bw/d (high dose, reduced to 120 mg/kg at week 12). No mortalities were seen in the low- and mid dose groups (mid dose 60 mg/kg bw/d). These doses corresponded to concentrations of 0.4%, 1.2% and 3.6/2.4% in corn oil for the low, mid and high dose groups, respectively. It is to be noted that effects at these concentrations would not lead to classification as skin irritant as the concentrations are below 5%.

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

All males and females of the high dose groups showed long-lasting piloerection from day 35 onwards. Ataxia was noted in one female. Reduced pupil size was detected in 3/7 male and 5/5 female survivors. Clinical abnormalities from the functional observational battery give some indications on abnormal neuromotor and sensory functions at 180/120 mg/kg bw/d. Gait impairment in one female and reduced pupil size (miosis – loss of capacity to adapt to darkness due to permanently contracted pupils) were seen in 3/7 males and 5/5 female survivors. The study authors interpreted these effects as being of unclear toxicological relevance that occurred at doses greater than the maximum tolerated dose (MTD).
The view of RAC is that a neurotoxic effect could not totally be excluded, as the effects were seen in surviving animals (after week 11) and miosis is not considered to be associated with gastritis. However, as the dose of 180 mg/kg bw/d during the first 11 weeks is above the guidance values for classification as STOT RE (100 mg/kg bw/d for a 90-day study), these effects do not warrant classification.

In principle, the mortalities at 180/120 mg/kg bw/d that occurred at day 49 or later could be relevant for classification for STOT RE, as they could not be seen as acute toxic effects. As the toxic effects at the high dose (including the ulcerative gastritis, peritonitis and a shift to higher relative numbers of neutrophilic granulocytes and reactive bone

marrow granulopoiesis) occurred at above the upper limit of the guidance values (100 mg/kg bw/d for a 90-day study), they do however not justify classification. Granulocytosis and increased granulopoiesis are likely to be secondary systemic effects to the chronic inflammantory and ulcerative processes in the stomach and peritonitis.

Local effects in the stomach were also observed in about half the animals (6 males, 5 females) treated at 60 mg/kg bw/d (1.2% in corn oil), increased medullary granulopoiesis was also seen in 4 males and 1 female at this dose. Repeated exposure to low concentrations that are not irritant at single exposure conditions may lead to exacerbations of adverse effects which over time may result in toxicologically significant effects. These chronic lesions could be relevant for classification for STOT RE. The DS argued that 60 mg/kg bw/d is more than half an order of magnitude lower than the dose mediating the acute toxicity and that the local effects are sufficiently be addressed by classification for corrosion/irritation.

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

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

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

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

For RP 3:2 the dose at which the effects in the stomach and bone marrow occurred in the 90-day study was much lower than the oral acute toxic doses (LD_{50} 630 mg/kg bw). The local effects in the stomach were not observed in three oral acute toxicity studies (at much higher test concentrations of 8% - 10%, highest dose tested 2000 mg/kg bw). RAC, in line with comments received from some MSCA during the public consultation, does not agree with the DS view that the local irritant effects are mechanistically sufficiently addressed with the classification for corrosion and should not support the classification for STOT RE.

The toxic effects in the GI tract are considered as chronic toxic effects that resulted from prolonged/repeated exposure to low concentrations/doses of RP 3:2. The effects are considered as reflecting repeated exposure toxicity and not just acute toxicity. Because they occurred within the range of guidance values (CLP regulation, Table 3.9.2-a, ≤ 100 mg/kg bw/d for an oral 90-day study) and the effective dose is considerably lower than the acutely toxic dose, RP 1:1 should be classified for STOT RE. Local effects in the GI tract (like chronic oesophagitis, gastritis) after repeated/prolonged exposure are toxicogically relevant as they impair not only the morphology and/or function of the locally targeted organ, but also bear the potential to impair adherent tissues/organs by transmural extension of the chronic inflammation (e.g. peritonitis, pleuritis) or to cause delayed mortalities (after ulceration into body cavities). Thus, RAC propose to classify RP 3:2 as STOT RE 2; H373 - May cause damage to (gastrointestinal tract) through prolonged or repeated exposure.

Formaldehyde
There is no harmonised classification on formaldehyde for STOT RE.
Lesions related to the irritancy in the stomach are - similar to RP 3:2 - the main effects after repeated oral administration of formaldehyde. However, available studies suggest that the lesions were seen at comparatively higher doses or occurred with lower severity grades compared to RP 3:2.

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

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

RP 1:1
The DS indicated the 90-day study of Lubrizol Deutschland GmbH (2002) to be of higher relevance than the second 90-day study of Schülke & Mayr (2002), both conducted in accordance with OECD TG 408.

In the first gavage 90-day study on 10 male and 10 female rats/dose groups that received 0, 12, 30, 80 or 150 mg/kg bw/d RP 1:1 (concentrations 0, 0.48, 1.2, 3.2 or 6% in peanut oil) (Lubrizol Deutschland GmbH, 2002), 2 males died after the first dose of 200 mg/kg bw/d which was then reduced to 150 mg/kg bw/d. Lesions in this region were found at this dose in both males and in 1 male that died at day 52 and in 1 female that died at day 75.

Abnormal breathing sounds were noted in animals at 80 mg/kg bw/d (1 female that died on day 68, 3 males (including 1 male which died on day 68 with pharyno-laryngeal lesions), at week 5 or later) and 150 mg/kg bw/d (4 males, 3 females starting at week 2). From the latter dose, 2 males and 2 females showed poor general condition and reduced activity. Reduced motor activity was observed in 1 female and 1 male at 30 mg/kg bw/d and in 1 female at 150 mg/kg bw/d.

Histopathology on animals which died during the exposure period revealed laryngitis in 1/1 male at 80 mg/kg and in 2/3 males and 1/1 female at 150 mg/kg bw/d, ulcerative laryngitis in 1/3 males at 150 mg/kg bw/d and pharyngitis in 1/3 males at 150 mg/kg bw/d and oesophagus lesions (mural inflammation and myopathy) in 3/9 females and mural inflammation only in 1/9 females at 150 mg/kg bw/d.
In surviving animals at 150 mg/kg bw/d, purulent rhinitis was observed in 1/7 males and 1/9 females and stomach submucosal inflammation in 1/7 males.

No treatment-related findings were seen at 30 mg/kg bw/d except in 1 female that died on day 38 with reduced activity, reduced skin turgor, reduction of bw and enlarged submandibular lymph node and 1 surviving female rat which showed nose bleeding, corneal opacity of a bloody left eye and a hairless region around the eye.

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

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

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

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

The pharynx/larynx was also examined in the second 90-day study (Schülke & Mayr, 2001). The DS interpreted this study as not valid as the MTD was not clearly reached and no local GI tract effects were seen. The absence of local effects in the upper GI tract after gavage administration with doses up to 180 mg/kg bw/d at concentrations up to 2.5% in water as the vehicle, may be related to the less concentrated test material and/or to the lack of maladministration.
No treatment-related mortality was observed at 0, 40, 100 or 250 mg/kg bw/d. High dose females showed a decreased motor activity (measured). Food consumption was significantly lower in males of the mid and high dose group at week 11, a slight dose-dependent decrease in bw gain was seen during the last 3 weeks of the treatment period for the high dose males (-9%) and mid and high dose females (-8%). Several effects on haematology, clinical chemistry and organ weights were reported. However, the study seems to be of limited value due to varying degree of pneumonic changes with histopathological characteristic of mycoplasal pneumonia that was indicated in the study report according to a note of the Rapporteur Member State (RMS).

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

As no valid information on oral repeated dose is available on RP 1:1, read across on RP 3:2 is proposed based on the same constituents of UVCB at a slightly lower concentration of releasable formaldehyde (28% from RP 1:1 versus 45% formaldehyde from RP 3:2). The read across to RP 3:2 is in line with the argumentation in the RP 1:1 Doc IIIA, where the applicant suggested using the data on RP 3:2.

Consistent with RP 3:2, RAC proposes to classify RP 1:1 as **STOT RE 2; H373 - May cause damage to (gastrointestinal tract) through prolonged or repeated exposure.**

Dermal route

RP 3:2
No repeated dose study using the dermal route is available.

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

2-Hydroxpropylamine
No repeated dose study using the dermal route is available.

RP 1:1
No repeated dose study using the dermal route is available.
Taking the data from formaldehyde into account, the overall database is not sufficient to take any decision on classification for STOT RE for this route.

Inhalation route

RP 3:2
No repeated dose study using the inhalation route is available.

Formaldehyde
Due to the lack of data on RP 1:1, data on formaldehyde were assessed for STOT RE:

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

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

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

In addition, Section 3.9.2.5.1 gives guidance on the relevant doses

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

the acute toxicity."

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

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

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

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

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

From repeated dose inhalation studies in animals, dose-dependent non-neoplastic lesions in the nasal cavity that increased in severity and extension with exposure time and dose (for review see SCCS 2014; BfR, 2006) were reported. Following inhalation exposure up to 24 months, squamous metaplasia was observed in rats at 6 ppm formaldehyde. Epithelial hypertrophy, hyperplasia and metaplasia, mixed inflammatory cell infiltrates, turbinate adhesions were seen at 10 ppm, in addition destructed turbinate architecture occurred at 15 ppm (Monticello et al., 1996, cited from BfR, 2006). While lesions of the respiratory epithelium in the nasal cavity were not reported after 6 weeks exposure up to 2 ppm (Monticello *et al*., 1991; Formaldehyde Core document IIIA), inhalation exposure of ≥12 months to ≥2 ppm (2.456 mg/m³) formaldehyde caused purulent rhinitis, epithelia dysplasia and squamous metaplasia at level I of the nasal cavity (Kerns *et al*., 1983 a, b, cited from BfR, 2006). At concentrations above 2 ppm, lesions extended to more posterior parts (level I to III) of the nose and reached the trachea at 14.3 ppm. Monticello (1989, cited from RAC Opinion on Formaldehyde) has demonstrated that inhalation of 6 ppm formaldehyde for 1 or 6 weeks induced loss of cilia, inflammatory response, epithelial hyperplasia and squamous metaplasia and increased cell proliferation in the nasal passages of Rhesus monkeys. Like in rats, lesions in monkeys showed an anterior-posterior gradient and duration-related increase in severity and extension, but these were more widespread than in rats. Inhalation of 3 ppm formaldehyde over 26 weeks induced squamous metaplasia and hyperplasia in the nasoturbinates in 6/6 Rhesus monkeys, while no effects were observed at 0.2 and 1 ppm (Rusch *et al*., 1983, see SCCS, 2014).

Taking 2 ppm formaldehyde as a robust LOAEC for chronic inflammatory and meta/hyperplastic lesions secondary to initial cytotoxicity in the nasal mucosa from repeated/prolonged inhalation and using the Haber's rule standard extrapolation from 12

months to 90-day exposure to compare with the guidance values, 2 ppm for 12 months corresponds to 8 ppm (9.824 mg/m³ = 0.01 mg/L) in a 90-day study. This is clearly below the guidance concentration of 50 ppm and would justify a classification of formaldehyde as STOT RE 1.

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

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

RP 1:1
No repeated dose study using the inhalation route is available.

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

Referring to the CLP Regulation, 3.9.2.10.3, RAC agrees with the DS on the read across to formaldehyde as data on repeated inhalation toxicity of RP 1:1 are lacking. However RAC does not agree that effects from repeated inhalation are covered by the classification for corrosion.

The absence of an entry for formaldehyde for STOT RE in CLP, Annex VI does not by itself justify non-classfication for RP 1:1.

The DS informed that RP 1:1 contains about 28% releasable formaldehyde. Assuming that under prolonged inhalation exposure conditions RP 1:1 would continuously release the maximal releasable amount of 28%, a factor of 3.6 should be applied to correct for the content of releasable formaldehyde.
As the human AEC was based on eye irritation, an acute receptor-mediated sensory irritation effect (without obvious cytotoxicity and infiltration of inflammatory cells) as surrogate for the lowest adverse effect in humans, animal data on repeated inhalation toxicity may be more appropriate to conclude on the classification for STOT RE.

For RP 1:1, the LOAEC for repeated inhalation exposure is based on the formaldehyde LOAEC of 2 ppm (2.456 mg/m³) derived from a rat 12-month study (Kerns *et al*., 1983 a,b) which would correspond to 8 ppm (9.824 mg/m³ = 0.01 mg/L) in a 90-day inhalation study based on Haber's rule. The 8 ppm LOAEC, corrected for the maximal amount of releasable formaldehyde (28%) from RP 1:1 with a factor of 3.6, results in a (corrected) concentration of 0.036 mg/L for RP 1:1 which is close to the lower boundary of the guidance value for STOT RE 2 ($0.02<C \leq 0.2$ mg/L). As inhalation exposure to the aerosol is expected to be the main concern for RP 1:1, the guidance values for the gaseous form were not considered.

If the chronic toxicity occurred at the same dose level as the acute inhalation toxicity, chronic toxicity would be covered by the classification for acute toxicity. The inhalative LC_{50} was unknown for RP 1:1 (and RP 3:2) as no acute inhalation study is available. As a substitute information on the difference between the level of the inhalation LC_{50} and the LOAEC for chronic effects for formaldehyde is considered. The Formaldehyde Core Document indicates an LC_{50} of 0.6 mg/L (4 h) which is markedly higher than the LOAEC for chronic effects (2 ppm = 2.456 mg/m³). Thus, the acute toxicity classification does not cover the classification for STOT RE.

It is noted that the formation of formaldehyde as hydrolysis product may depend on several factors (e.g. temperature, pH, dilution). The RMS raised uncertainties (Doc II-A

2.12) that exposure conditions or hydrophobic formulations may reduce the rate of hydrolysis, but may theoretically enhance deeper respiratory tract exposure and may also increase irritation properties due to their effect on membranes.

Repeated inhalation exposure to RP 1:1 generates the hydrolysis products formaldehyde and 2-hydroxypropylamine. Whether 2-hydroxypropylamine may exert additive effects to those expected from formaldehyde, remains unknown.

Based on the read across from data on formaldehyde (see above), RAC proposes to classify RP 1:1 with regards to target organ toxicity from repeated inhalation as STOT RE 2; H373 (May cause damage to the respiratory tract through prolonged or repeated exposure).

All routes/Overall classification on STOT RE
When classification for STOT RE is proposed based on data from several routes with different target organs, the final labelling should consider all the relevant target organs. RAC agrees that classfication of RP 1:1 is warranted as **STOT RE 2, H373 – May cause damage to the respiratory tract and the gastrointestinal tract through prolonged or repeated exposure.**

No specific route should be indicated.

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

4.8.1.1 In vitro data – 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.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier submitter's proposal
The DS proposed to classify RP 1:1 as a caterory 2 mutagen based on the existing harmonised classification of its hydrolysis product formaldehyde. There are several mutagenicity studies *in vitro* and *in vivo* for RP 1:1. Predominantly clastogenic effects are induced in cells of mammalian cell cultures whereas bacterial gene mutations tests are weakly positive (one test) or negative (one test). Regarding the *in vivo* testing, a negative result was obtained in an *in vivo* chromosomal aberration test after repeated gavage exposure to RP 1:1. After single i.p. injection of RP 1:1 an *in vivo* micronucleus test was negative whereas an *in vivo* chromosomal aberration test was positive. The positive result seems to be of questionable relevance due to deficiencies in the study (e.g. no statistical evaluation of the data). The DS additionally provided information on similar results of *in vitro*/*in vivo* mutagenicity tests for the substance RP 3:2. (To avoid a confusion it should be noted

that the corresponding references in the Tables 4.8-2 and 4.8-5 were taken from the CLH report for RP 3:2. They are not part of the reference list of the CLH report for RP 1:1.)

The DS also argued that due to the rapid hydrolysis of RP 1:1 to formaldehyde at contact with biological tissues, an induction of local genotoxic effects is to be expected at the site of first contact *in vivo*. Therefore the DS referred to the classification of formaldehyde, classified as Muta. 2, based on the induction of genotoxic effects *in vivo* on somatic cells at site of contact and supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. The other hydrolysis product 2-hydroxypropylamine is of very minor toxicological relevance.

Due to the mechanistic considerations of formaldehyde release from RP 1:1 the DS proposed to classify the substance RP 1:1 as a Muta. 2 on the basis of its hydrolysis product formaldehyde.

Comments received during public consultation

One MSCA expressed support for the proposed classification. One individual disagreed with the proposed classification as a category 2 mutagen due to the lack of relevant mutagenicity data.

Assessment and comparison with the classification criteria

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

Formaldehyde
RAC agrees with the approach of the DS to take into account the classification of formaldehyde as a category 2 mutagen for justification of the classification of RP 1:1.

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

RP 1:1
The evaluation of the mutagenicity data of RP 1:1 by the DS and RAC does not differ. RAC also comes to the conclusion that a proposal for classification of RP 1:1 as category 2 mutagen is justified.

In vitro data
The available bacterial gene mutation tests are weakly positive with S9-mix (Lubrizol Corporation, 2000, see Doc III A6.6.1/02) or negative (Schülke and Mayr, 2000, see Doc III A6.6.1/01). The negative results are not conclusive because the tested concentrations were below the highest concentration (5000 µg/plate or relevant cytotoxic concentration) recommended by the respective test guideline.
Two mouse lymphoma assays (Lubrizol Corporation, 2001 see Doc III A6.6.3/01; Schülke and Mayr, 2002, Doc III A6.6.3/02) are positive with and without S9-mix. In the analysis of the colony sizes predominantly small colonies were found, which indicate clastogenic activity of RP 1:1.
A chromosomal aberration test was positive in CHL cells with and without S9-mix (Lubrizol Corporation, 2001, Doc III A6.6.2).

In vivo data
Three studies are available that are able to detect systemic chromosome mutagenic

activity in bone marrow cells of mice.

An *in vivo* chromosomal aberration was negative after repeated oral administration (gavage) of RP 1:1 up to the highest tested dose of 425 mg/kg bw (Schülke and Mayr, 2000 (DocIIIA6.6.4/03)). Neither cytotoxic effects nor clinical signs were induced. Thus the highest tested doses did not correspond to the MTD nor was it in accordance with highest guideline recomended dose.

In two further *in vivo* tests RP 1:1 was injected once intraperitoneally. In a negative *in vivo* micronucleus test, the highest tested dose of 100 mg/kg bw induced cytotoxic effects (reduced PCE/NCE ratio) as well as minor clinical signs (Becker Chemie, 2002a (Doc III A6.6.4/01)). An *in vivo* chromosomal aberration test (Becker Chemie, 2002b (Doc III A6.6.4/02)) was positive at the highest tested doses of 50 and 100 mg/kg bw but the result seems to be of questionable relevance because there are deficiencies in the study (e.g. no statistical evaluation of the data).

The quantity of test data for RP 1:1 is limited and the available mutagenicity studies are not published. Thus, only the data given by the applicant in the biocide registration dossier are available. These data allow neither a detailed test evaluation nor they do allow to assess whether a test performance is fully in accordance with the corresponding guideline. But despite these limitations the following conclusion can be drawn: in bacteria as well as in somatic cell cultures mutagenic effects are induced. For RP 1:1 there is no reliable evidence for a systemic mutagenic effect. An *in vivo* micronucleus test in bone marrow cells of mice was negative after i.p. injection. The results of two *in vivo* chromosomal aberration tests are of limited relevance due to methodological deficiencies.

Formaldehyde, which is quickly released from RP 1:1 on contactwith biological tissues, is classified as a category 2 mutagen based on the induction of local genotoxic effects *in vivo* on somatic cells at the site of contact and are supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. Although it seems likely that the amount of formaldehyde may vary depending on different uses, the inherent potential of RP 1:1 to release formaldehyde is a critical factor.

Testing of the *in vitro* mutagenicity of RP 1:1 shows that the observed positive effects are consistent with those known from formaldehyde alone. Uncertainties remain due to the relevance of the available (negative) *in vivo* studies. However, it is assumed that RP 1:1 – like formaldehyde - has a poor systemic availability *in vivo* due to its rapid hydrolysis. Therefore it seems unlikely that genotoxic effects would be induced at a site distant from first contact.

Although no distinct criteria is noted on reaction products from UVCBs in the CLP Regulation, (likewise for CMR substances in mixtures, Art. 6.3 and 1.6.3.1 of the CLP Guidance) the information on the hydrolysis product is used to assess the mutagenic potential of RP 1:1.

RAC discussed that due to its reactivity, poor systemic availability is expected for RP 1:1 and therefore, the induction of systemic genotoxic effects is unlikely. However, a local genotoxic effect produced by the hydrolysis product formaldehyde is expected and RAC considers read across to formaldehyde (which is classified as a mutagen category 2 based on its local genotoxic action) justified. Some RAC members expressed the view that the guidance relates only to classification of substances that causes germ cell mutations. This view is reflected in a minority position supported by two RAC members. RAC recognised that according to the CLP Guidance, Section 3.5.1, classification is also warranted if there is evidence of only somatic cell genotoxicity that leads to classification in Category 2 if genotoxic substances are only acting locally.

RAC agrees with the proposal of the DS to classify RP 1:1 as a **Germ cell mutagen, category 2; H341 (Suspected of causing genetic defects)** based on the properties of its hydrolysis product formaldehyde.

Supplemental information - In depth analyses by RAC

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

RP 1:1 has low systemic availability due to its rapid hydrolysis. Accordingly, the available *in vivo* results are of low relevance because they examine a possible induction of mutagenic effects at sites distant from the site of exposure. Therefore their results do not allow to conclude that the substance is not genotoxic in the whole animal. There is no test with RP 1:1 assessing whether genotoxic effects will be induced in cells at the site of first contact. However, for the evaluation of the toxicological properties of RP 1:1 it is taken into account that its hydrolysis product formaldehyde is already classified as a category 2 mutagen due to the induction of local genotoxic effects.

4.9 Carcinogenicity

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

No long-term 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 → 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.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

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

Comments received during public consultation

Three MSCAs agreed and four industry commenters disagreed with the classification proposal. Some industry commenters suggested to classify RP 1:1 on the basis of the content of free (unbound) formaldehyde. Since the formaldehyde content is below 0.1% no classification was found to be justified.

Assessment and comparison with the classification criteria

RP 3:2
No carcinogenicity studies are available on RP 3:2.

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

2-Hydroxypropylamine

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

RP 1:1
There are no reliable human data. Two medical letter reports in Doc III A stated that no adverse effects have been documented from annual medical screenings that could be ascribed to employees in the manufacturing of products containing formaldehyde releasing biocides or with the active substance RP 1:1. No information is given on the details of the level, duration, frequency and conditions of exposure, on the substances the workers were exposed to or on the details of the medical examinations and results.

No studies on carcinogenicity or prolonged/repeated inhalation exposures are available for RP 1:1. The non-submission of data was justified by a read across to formaldehyde, and the probable carcinogenic effects of RP 1:1 are considered by the biocide applicant to be related to the hydrolysis product formaldehyde (Doc III A6.7).

It is expected that RP 1:1 exerts similar effects as formaldehyde such as cytotoxicity, hyperplasia, metaplasia, tumours and local mutagenic effects at the sites of contact - on the epithelium of the respiratory tract following prolonged inhalation - as formaldehyde is one of the main hydrolysis products.
It is assumed for RP 1:1 that, similar to formaldehyde, systemically increased bioavailability and a concern for systemic carcinogenic responses are not to be expected.

Although it is noted that the amount of formaldehyde released may vary depending on different uses, the reaction product of paraformaldehyde and 2-hydroxypropylamine is intended to release formaldehyde in aqueous solutions. RP 1:1 is expected to hydrolyse completely under aqueous environmental conditions or when the substance has entered the human or animal bodies (Doc II A1.4.3). Both hydrolysis products, formaldehyde and 2-hydroxypropylamine, are considered to be slightly volatile from aqueous solutions.

The following is presented as clarification of the objectives of the classification proposal and in response to some comments received during public consultation. Exposure to formaldehyde may result from inhalation or dermal exposure to RP 1:1 as an active substance. This can result from exposure to the undiluted UVCB substance and (as considered in the CLH report) the contact with biological tissues/media then generates hydrolysis products (including formaldehyde). Similarly, exposure to RP 1:1 in aqueous solution (such as diluted formulations or products on the market) can result in contact with hydrolysis products from the dilution and with those directly generated following contact with biological media. Coinciding with the above can be exposure to the gaseous form after evaporation of formaldehyde from the undiluted or diluted RP 1:1.

Formaldehyde is classified based on its carcinogenic potential at the sites of exposure, primarily on the nasopharyngeal tumours observed in man and rodents after prolonged inhalation[4].

The CLP Guidance, Section 3.6.2.2.7 states that:

> *"A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of*

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

other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes."

The CLP Guidance (section 1.4.3) explicitly foresees the read across of information from 'source' substances to predict the same hazard for another 'target' substance. For RP 1:1, it is not about the similarity of source and target substance, but RP 1:1 should be classified as a carcinogen based on the release of the *identical* substance (formaldehyde) resulting from hydrolytic transformation of RP 1:1.

Endpoints, on which data on RP 1:1 are available, show that effects were consistent with those known from formaldehyde alone. With regards to the oral repeated toxicity, with the observation that the toxicity may be more severe for RP 3:2 when comparing the dose levels or the severity of effects observed with formaldehyde, no valid study was available for RP 1:1. However uncertainties remain due to the lack of studies with full guideline compliance and as an additional contribution of the other hydrolysis product 2-hydroxypropylamine to the effects by formaldehyde are unknown.

As mentioned by the DS, from a quantitative aspect, the hydrolysis rate of RP 1:1 to formaldehyde depends on several environmental factors (increase at higher temperature, lower pH, and at higher dilution). At all tested pH levels the hydrolysis half-life was less than 1 h. However, water contact or dilution of RP 1:1 with aqueous solutions are not a necessary condition for exerting toxic effects of RP 1:1, for the aerosol aqueous conditions were given at contact sites (mucous membranes with oral & inhalation exposure, sweaty skin with dermal exposure) and as demonstrated by similar toxic effects with lipid vehicles. The CLH report stated that the equilibrium of RP 1:1 (or RP 3:2) shifts towards formaldehyde (by dilution and) by the reaction of formaldehyde with biological media.

With regards to the Industry representative comments during public consultation that a classification of RP 1:1 as a carcinogen is not justified based on the end use 'diluted metalworking fluid' containing less than 0.05% of 'free, unbound' formaldehyde and the slow rate of formaldehyde release during its use, the DS replied that the uses and different dilutions that are on the market are not relevant for the decision on the hazard of the substance itself.

The Industry representative stated that evidence is lacking that sufficient formaldehyde will be released during exposure to workers to cause a carcinogenic risk. RAC considers the lack of observations in annual medical screenings of the type presented here not to be robust information. RAC notes that the CLP Regulation states that a classification is based on the intrinsic hazards of a substance and does not take the exposure conditions and the exposure to mixtures containing the substance of concern into account.

The option to classify RP 1:1 as a carcinogen in category 2 in order to account for uncertainties for substances that are unstable, showing equilibrium behaviour and having half-lives depending on dilution, temperature and pH as discussed in the CLH report is not supported by RAC. By weighing the evidence from read across to the specific substance (and hydrolysis product) that is known to have carcinogenic properties (formaldehyde), no reasons (such as uncertainty about structural similarity or qualititative differences in the mechanistic aspects) could be identified to justify category 2 and RAC considers that the data supports category 1B. Hydrolysis tests indeed have demonstrated that high concentrations of formaldehyde are generated within short time periods.

These hydrolysis tests support qualitatively that hydrolysis will occur in contact with aqueous biological media on mucous membrans. Inhalation exposure to aerosolic RP 1:1 is expected to result in hydrolysis at the site of contact and toxicologically significant

concentrations of formaldehyde could be reached on the surface of the mucous membranes in the respiratory tract, eye or upper GI tract or skin. The inhalation exposure to gaseous formaldehyde that evaporated from RP 1:1 is assumed to contribute in addition to the toxic/carcinogenic effect resulting from the direct impact of hydrolysis products at the contact site. Demonstrating that the room concentrations of released gaseous formaldehyde are rather low would not be sufficient to discount the hazardous potential that may result from the aerosol exposure to RP1:1.
As no data are available to demonstrate that a sufficiently high concentration of formaldehyde cannot (meaning: has not the potential to) be reached, there is no evidence to justify a lower classification. This prerequisite of evidence, and the fact that CLP is hazard based, is in contrast to the opinion of some commenters during public consultation, who argued that the classification is only justified if evidence from exposed workers demonstrates that sufficient formaldehyde will be released and have caused tumours.

Although no specific mention is made on classification of reaction products from UVCBs in the CLP Regulation, (likewise for CMR substances in mixtures, Art. 6.3 of the CLP Regulation and section 1.6.3.1 of the CLP Guidance) information on the hydrolysis product is used here to assess the hazardous properties including the carcinogenic potential of RP 1:1. More guidance is given in REACH, Annex XI, 1.5.2 that specifies that similarities to substantiate the read across may be based on common precursors or common breakdown products via physical or biological processes, which results in structurally similar chemicals.

RAC agrees with the proposal of the DS to classify RP 1:1 as **Carc. 1B; H350 (May cause cancer)**.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information – RP 1:1

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

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.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

No reprotoxicity study are available for RP 1:1. For RP 3:2 there is one developmental (OECD TG 414) and one fertility study (OECD TG 415). The resuls of these studies do not support classification for either sexual function and fertility or development, according to the DS.

Comments received during public consultation

One MSCA requested further information and argumentation.

Additional key elements

RP 3:2

Effects on fertility

The documentation of the results of the 1-generation study on RP 3:2 in the CLH report on RP 3:2 was not sufficiently detailed to enable a conclusion to be reached on whether the fertility effects are direct or secondary effects or whether or not classification is justified. More details from the RP 3:2 Doc III A and from the original study report are reported below.

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

day 21 (females).

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

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

In female rats, no effects were reported at 15 mg/kg bw/d. One high dose dam (No. 185) showed lethargy, diarrhea and dystocia. Female rats at this dose (45 mg/kg bw/d) showed slightly (non-significantly) reduced food consumption during the last gestational week (-6.3%). Mean body weight (and bw gain) was not affected. Stomach lesions were macroscopically noted in 2 out of 24 females of the high dose group. Microscopically minimal to moderate squamous hyperplasia (5 rats) with hyperkeratosis (7 rats), submucosal inflammation (2 rats) and edema (1 rat) were observed in females of this dose group. The evaluation of the RMS (in MBO Doc III A6.8.2_1) in agreement with the individual animal data of the study report outlines that 4/24 females showed ulcerations of the forestomach.

In their evaluation, the RMS (MBO Doc III A6.8.2_1) also noted that '*additionally females were observed for signs of difficult or prolonged parturition, and behavioural abnormalities in nesting and nursing*' without giving information on the dose groups affected. No information related to the clinical abnormalities around parturition could be found in the summary of the study in MBO Doc III A6.8.2_1 and the study report (except the one case with dystocia (No. 185)).

No effect was seen on the fertility index (no. of pregnant females/no. of females cohabitated) (87.5% in controls, 91,7%, 87.5% and 91.7% in low, mid and high dose groups, respectively).

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

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

No treatment-related effect on pup survival was seen at day 4. The high post-natal loss until

day 4 in the control group (27 dead pups, 10.9%) was noted by the RMS in the MBO Doc III A. The original study report indicated that the 26/27 mortalities in the control pups stem from 3 litters (Nr. 109 10/13 deaths on day 2; No. 112 12/13 deaths on day 2 (of which 9 were missing), 1/13 death on day 1; No. 120 3/11 missing on day 2, 1 death on day 1). Additional information indicated that the mean viability index of historical controls in 17 control groups was 98.9% (it was assumed that this information came from the the same laboratory, no information on the years when these were conducted). This study was found to be within the historical control range, as one out of the 17 studies revealed also a viability index of 89.1%.
In addition, no clear-dose relationship was found on the pup survival of treated groups from day 1 to day 4 (if considered separately from survival at day 0/1) when comparing the no. of dead pups in the low, mid and high dose groups (4%, 2.9% and 7.9%).

Table: Summary on maternal toxicity (corresponds to the upper part of Table A6_8_2-2 MBO Doc III A6.8.2_1.)

			control		**low dose**		**medium dose**		**high dose**	
Parameter		Generation	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	incidence	**P**	0	0	0	0	0	0	0	0
Food consumption (prepairing period, days 1-70)	% of control	**P**			- 1.5	+1.5	-1	+2.9	-5.8	+1.5
Food consumption (females, last gestational week, day 14-21)	% of control	**P**				+5.2		+2.9		-6.3
Differences in mean body weight gain (prepairing period, from day 1 to 70)	% of control	**P**	+107	+55	+103	+54	+107	+56	+101	+57
Clinical Observations	Incidence	**P**	n.s.f.	n.s.f	n.s.f.	n.s.f.	n.s.f.	n.s.f.	n.s.f.	1#
Pathology		**P**								
Macroscopic findings (Stomach)		**P**								
Cratiform retractions	Incidence, (%)	**P**	0	0	0	0	0	0	9(38)	1(4)
Isolated cratiform retractions	Incidence, (%)	**P**	0	0	0	0	0	0	3(13)	1(4)
Nodules	Incidence, (%)	**P**	0	0	0	0	0	0	5(21)	0
Several cratiform retractions	Incidence, (%)	**P**	0	0	0	0	0	0	1(4)	0
Histopathologic examination (Forestomach)										
No. animals examined		**P**	24	24	24	24	24	24	24	24
Ulcerations	Incidence*	**P**	0	0	0	0	0	0	19 (4.1)	4 (3.3)
Vacuolar degeneration	Incidence	**P**	0	0	0	0	4 (1.8)	0	5 (1.6)	0
Squamous hyperplasia	Incidence	**P**	0	0	0	0	5 (1.0)	0	10 (1.5)	5 (1.2)
Hyperkeratosis	Incidence	**P**	0	0	1 (2.0)	0	5 (1.0)		18 (1.4)	7 (1.0)
Submucosal inflammation	Incidence	**P**	0	0	0	0	2 (1.0)	0	4 (1.3)	2 2.5)
Submucosal oedema	Incidence	P	0	0	0	0	1 (1.0)	0	2 (2.0)	1 (2.0)

n.s.f.: no specific findings * (mean severity)#: one high-dosed female showed lethargy, diarrhea and dystocia and had cannibalised its pups

Table: Summary on reproductive performance (corresponds to the lower part of Table A6_8_2-2 MBO Doc III A6.8.2_1.)

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

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

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

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

In the CLH report, the increased implantation losses and the increased incidences of pup death were reported only as a sum (without giving further quantitative information). The lack of separate documentation on these effects was justified in a separate assessment document (MBO Doc III A6.8.2-2) by the argument that it could not be determined whether there had been post implantation loss or post natal loss, where pups were cannibalised directly after birth by its mother. The author of the assessment document found it prudent to calculate the number of post implantation losses plus the number of postnatal losses between day 0 and 4 as the combined incidence based on separate incidences and to reflect only on this combined incidence for the overall conclusion. The overall numbers of the sum were reported as 47, 45, 48, 66 in the control and dose groups.

In addition, the individual study findings were summarised by the RMS in a table (Table 4 of the MBO Doc III A6.8.2-2) indicating that numbers of pups could not be reported for 2 out of 24 dams. This means that the numbers of living animals after birth and at day 4 are available for 22 dams (as documented in the Table A6_8_2-2 (see above)).

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

Table: Summary on implantation loss and pup viability
(extracted from MBO Doc III A6.8.2-1 and 6.8.2-2)

Groups Effects	Control	5 mg/kg bw/d	15 mg/kd bw/d	45 mg/kg bw/d
Implantation sites	268	285	279	288
Implantation loss/number§	20	**35***	**41****	**47****
Implantation loss/%	7.5	12.3	14.7	16.5
§Thereof: Dead pups at first litter check (Day 0/1)	3	2	12	24
Live pups at Day 0/1	248	250	238	241
Post-natal loss Day 0-4 (%)	27# (expected 2.7) (10.9)	**10**** (4.0)	**7**** (2.9)	19 (7.8)
Live pups at Day 4	221 (expected 242.3)	240	231	222
% of live pups related to numbers of implantation site	82.5 (expected 90.4)	84.2	82.3	77

Abnormal high spontaneous pup deaths until day 4, expected value based on historical control data on mean survival of 98.9% * / **: Fisher's Exact Test significant at 5% (*) or 1% (**) level

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

- There is no indication from the 1-generation study on RP 3:2 that male fertility was affected.

- There is no indication of severe systemic toxicity in the females during and at the end of the treatment period. The slightly reduced food consumption of high dose females during the last gestational week only (-6.5%) did not affect the body weight. In high dose male rats at the end of the pre-pairing period (day 70), the food consumption was slightly lower (-5.5%) than in controls.

- The total numbers of implantation loss and the implantation losses per litter were dose-dependently increased in the low, mid and high dose groups.

- In 10/22 dams of the high dose group implantation losses were seen; only in 4 out of 24 paired female rats, ulcerations of the forestomach were reported (in 3 out of 22 evaluated dams; 2 out of 24 dams cannibalised their pups, 1 of these showed a moderate ulceration of the stomach (No. 185)). Its severity is reported as moderate to marked (the information on the grading is lacking in the CLH report and the summary Table A6_8_2-2, but can be found in MBO Doc III A6.8.2-2 p.7ff and in the study report). Less severe stomach effects such as squamous hyperplasia (in 5 rats), hyperkeratosis (in 7 rats) and submucosal inflammation (in 2 rats) reported as minimal to moderate, were also observed in dams of this dose group.

- The 2 dams (No. 178, 185) that cannibalised their pups were not included in the evaluation of the reproduction parameters.

- Whether the increases in implantation losses and dead pups occurred in litters from dams with forestomach lesions, is not documented in the CLH report. The RMS analysed individual findings (Page 7ff of MBO Doc III A6.8.2-2): 4 of 24 pregnant

females had ulcerations of the forestomach. The implantation losses in three of them were 1 of 8 implantation sites, 0 of 14 implantation sites and 13 of 15 implantation sites (Animal no. 179, 180, 190 of 22 dams evaluated). One dam (no. 185) with ulceration cannibalised its pups (not considered in the evaluation, Table A6_8_2-2).
With regard to the implantation losses, the historical control in the the same laboratory were reported from 17 studies with a median of 7.9% (range 1.6% to 12.2%, no data on the years when the study was conducted). The control group in the 1-generation study corresponded well to the median value of the historical controls, meaning that 0.96 implantation losses per litter may be considered normal. Considering the number of dams with 2 or more implantation losses out of 22 dams, there was no effect on the stomach in 4 dams with increased implantation losses (no. 169, 175, 184, 192) and minor effects such as hyperkeratosis and/or squamous hyperplasia (effects that primarily protect against irritation) were seen in two dams with increased implantation losses (no. 173, 176). In conclusion, increased implantation losses were seen in dams without effects on the forestomach, in dams with slight effects in the forestomach and in 1 out of 4 dams with ulceration of the forestomach. This distribution does not support the conclusion that severe (ulcerative) effects of the forestomach were causally related to the implantation losses.

- In the low and mid dose groups the implantation losses occurred without *any* effect on the stomach or *any* other sign of systemic toxicity.

- Pup survival at the first litter check (day 0/1) was dose-dependently reduced in the mid and high dose groups. No indication on maternal toxicity was seen in the mid dose dams.

- Post-implantation losses and reduced pup survival at the low and mid-dose levels can not be attributed to secondary effects.

- No treatment-related effect on pup survival from day 1-4 was observed. A dose-response was less clear for this period. Increased post-natal losses were seen in the control and high dose groups and significantly lower day 1-4 deaths were seen at the mid and low dose group. An unusually high rate of pup deaths was seen in the control groups due to high numbers of mortalities in 3 litters on day 2. This finding may have caused the low relative to the control death rates at the low and mid dose levels and may have masked a treatment-related effect at the high dose, but these assumptions could not be verified as no causalities were identified. Thus, effects on pup survival on day 0-4 were uncertain and were not taken into account for classification purposes.

- Increased post-implantation losses and reduced pup survival at first litter check (day 0/1) were considered related to the administration of RP 3:2.

- On the individual animal level, the implantation loss was not associated with ulcerative lesions of the forestomach in dams. Both effects occurred also at doses without stomach lesions or any other systemic toxicity. Thus the effects are considered direct and not secondary effects.

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

Developmental toxicity

In a developmental toxicity study conducted according to OECD TG 414, rabbits were gavaged with 0, 5, 35, 90 or 135 mg/kg bw/d RP 3:2 in corn oil. A dose of 135 mg/kg bw/d resulted in

severe maternal toxicity (decrease in food consumption (GD 11-14, GD 28), lower body weight (max. -9%), reduction in bw increase (GD 9-12, 18-21, 27-29, no quantitative data) increased mortality (10 pregnant rabbits between GD 9-23, 1 non-pregnant rabbit at day 17, 4 dams sacrificed after abortions).

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

Total implantation loss was seen in 3 out of 22 dams at 135 mg/kg bw/d, in 1 out of 24 dams at 5 mg/kg bw/d (No. 30), 1 out of 22 dams at 90 mg/kg bw/d (Nr. 120). Developmental toxicity findings such as an increased number of early and late resorptions, a decreased number of foetuses, an increase in post-implantation losses and mortality of foetuses was only observed at 135 mg/kg bw/d.

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

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

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

Macroscopic findings	Control n = 23 #	5 mg/kg bw/d n = 24 #	45 mg/kg bw/d n = 23#	90 mg/kg bw/d n = 21 #	135 mg/kg bw/d n = 22 #
Stomach:					
mucosa: detachment (and reddening)	1 #	2 #	1 #	0	10 #
reddened	0	0	3	3 #	10#
ulcers (a few, multiple)	0	2 #	1	0	4 #
haemorrhagic/dark/reddish/black	0	0	1	4 #	8 #
distension	0	0	0	0	3
Large Intestine:					
reddened	0	0	1 #	1 #	0
Duodenum:					
reddened	0	0	0	0	2 #
Kidney:					
dilatation of renal pelvis	1	4 #	4 #	4	12 #
cysts (medulla, cortex)	0	1	1	0	0
coarse structure (cortex)	0	0	2	0	2 #
very soft	0	0	0	0	1 #
Spleen:					
reduced in size	0	0	1	3 #	1
Liver:					
pale (with dark foci)	0	0	6	3	2
glossy (light and dark)	0	0	0	0	1

Lungs:					
right lobe reddened	0	0	0	0	1 #
Vagina:					
reddish mucus	0	0	0	0	2

(including) prematurely decreased animal(s).

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

	Control	**5 mg/kg**	**45 mg/kg**	**135 mg/kg**	**r90 mg/kg**
Treated dams	24	24	24	24	24
Non-pregnant dams	1[12]	0	1[12]	1[12]	2
Dams with abortion	2	1	2	4	1
Dams without viable fetuses	0	1[13]	0	3[13]	1[13]
Prematurely deceased dams (pregnant and non-pregnant)	2[12]	2	2[12]	11[12]	3
Not examined dams	0	0	0	1[14]	1[14]
Evaluated litters	**20**	**20**	**20**	**5**	**16**

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

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

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

Assessment and comparison with the classification criteria

RP 3:2

Effects on fertility

From the available data on repeated dose studies and a 1-generation study with 70 days of premating treatment, no indication is given of adverse effects on the male sexual function or fertility of the male rat.

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

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

Developmental toxicity

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

increased, e.g. higher than 10%.

Based on the available data RAC concludes that no classification is warranted for developmental effects.

Formaldehyde

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

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

2-Hydroxypropylamine
No data on fertility and developmental toxicity available.

Effects on fertility
No studies on sexual function and fertility are available on RP 1:1.

RP 3:2 hydrolyses to the 1:1 reaction product, the data on RP 3:2 are relevant for RP 1:1.

From the available data on RP 3:2 on repeated dose studies and a 1-generation study with 70 days of premating treatment no indication is given on adverse effects on the male sexual function or fertility of the male rat. In addition no concern was identified for RP 1:1 from the available 90-day studies that were of limited validity.

Based on the same hydrolysis products (although with a lower maximum concentration of releasable formaldehyde) read across to the 1-generation study on RP 3:2 is proposed.
RAC concludes for RP 1:1 that **no classification is warranted for fertility effects**.

Developmental toxicity
No developmental studies are available on RP 1:1.

RP 3:2 hydrolyses to the 1:1 reaction product, the data on RP 3:2 are relevant for RP 1:1.

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

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

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

.

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

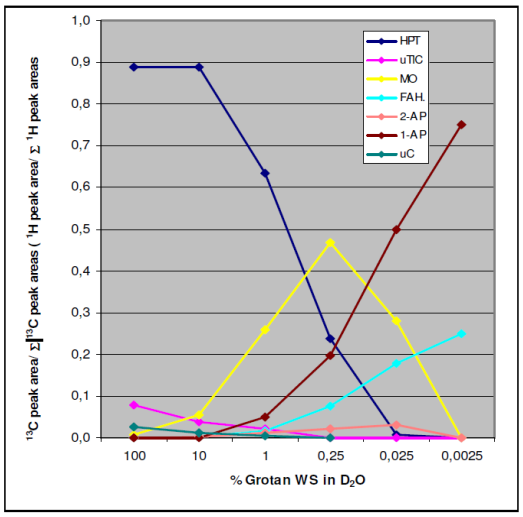
5.1.1 Stability

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)
uTlC: unidentified triazine-like compound
MO: 5-methyloxazolidine
FAH: formaldehyde hydrate
2-AP: 2-aminoproanol(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-aminoropanol(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[5]) 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

[5] 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[6] 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^{TM} 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[7] 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.

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

[7] 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)[8] 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.

[8] 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[9]. From the presented data the two reaction products show comparable toxicity

[9] 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* Grotan® 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)[10] 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)[11], 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).

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

[11] 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 Grotan® 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^{TM} 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:

➔ **No classification**

Aquatic Chronic 2:

➔ **No classification**

Aquatic Chronic 3:

➔ **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).	

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier submitter's proposal The substance "Reaction product of paraformaldehyde and 2-hydroxypropylamine (ratio 1:1)" (RP 1:1) is a formaldehyde-releasing UVCB substance with bactericidal and fungicidal properties and is employed as a biocidal active substance.

Degradation

The dossier submitter proposed to consider RP 1:1 as rapidly degradable because one out of two studies on ready biodegradability (both performed according to the OECD TG 301D (Closed-Bottle-Test)) showed 62.7% degradation after 28 days.

Aquatic Bioaccumulation

According to the dossier submitter, RP 1:1 does not meet the CLP criteria for bioaccumulation. There are no experimental data on bioaccumulation of RP 1:1 available, however, based on the hydrolysis products formaldehyde ($logK_{ow}$ = 0.48) and 2-hydroxypropylamine ($logK_{ow}$=0.61) the potential for bioaccumulation of RP 1:1 was considered low.

Acute Toxicity

The dossier submitter proposed to not classify RP 1:1 as acutely hazardous to the aquatic environment. The basis for this proposal is that $L(E)C_{50}$ values for all three trophic levels are >1 mg/L and the lowest $L(E)C_{50}$ value was derived for algae with E_rC_{50} = 2.9 mg/L.

Chronic Toxicity

The dossier submitter proposed to classify RP 1:1 as Aquatic Chronic 3 (H412) based on rapid degradability and the lowest chronic toxicity in algae (*Pseudokirchneriella subcapitata*, E_rC_{10} = 0.148 mg/L). Algae have been shown to be the most sensitive throphic level in aquatic acute toxicity tests. The NOEC was determined in the study report (Dunett´s test using individual replicate values) to be <0.05 mg/L. Based on the flat dose-response curve and the observed difference (factor of 9) between biomass and growth rate, an E_rC_{10} of 0.148 mg/L (corrected for 76% recovery) was derived. In a second study on algae (*Desmodemus subspicatus*) a 72h-NOE_rC of 0.9 mg/L was derived and supports the proposed classification. For *Daphnia* no chronic study is available, however a read-across to the chronic *Daphnia* study with RP 3:2 resulted in a NOEC of 1.3 mg/L based on mean survival of the offspring. For fish no chronic study is available.

Comments received during public consultation

Three MSCAs commented on the environmental hazards and one of them supported the dossier submitter's proposal.

One commenting MS questioned the scientific quality of the study on ready biodegradability and wether it was carried out under GLP. Also the use of potassium hydrogen phthalate as a reference substance and the use of river water as the inoculum were questioned and noted that it is not possible to rule out adaption to the test item. The same commenting MS highlighted that for the two hydrolysis degradation products 2 –hydroxypropylamine and formaldehyde it is unclear if additional data for an environmental hazard classification are available.

Another commenter requested further explanations on the hydrolysis and on the degradation of the hydrolysis products.

Assessment and comparison with the classification criteria

Degradation

RAC notes that degradability tests on UVCB substances may only be considered relevant if it has been shown that the UVCB substance only contains structurally similar constituents that are expected to behave in the same way in the tests. RAC in general prefers the assessment of degradation via a testing approach where relevant constituents

of a UVCB substance are first subjected to screening assessment individually. If certain constituents represent the worst case with regard to degradability, these "defined constituents" may be used for further testing and for assessing the entire UVCB substance.

Ready biodegradability

The potential for biotic degradation of RP 1:1 was investigated in two studies on ready biodegradability both performed according to the OECD TG 301D (Closed-Bottle-Test). In one test the pass level for ready biodegradability was clearly not reached. It was also shown that RP 1:1 was not toxic to microorganisms. Both hydrolysis products formaldehyde and 2-hydroxypropylamine on their own seem to be readily biodegradable. Consequently, the observation that the UVCB substance RP 1:1 is not readily degradable may indicate that it might include constituents which biodegrade at a slower rate or biodegradation products are formed which degrade more slowly. The fact that the 10-day window is not fulfilled may indicate that some compounds are not readily degradable. Overall no further explanation of the negative test result is given which would question the reliability of the study for classification purposes.

The second test showed 62.7% degradation after 28 days which is close to the threshold criterion in CLP. The dossier submitter rated this study with Klimisch score 2. One commenting MSCA CA questioned its scientific quality and reliability. RAC notes that the study appears to have been carried out under GLP. Adaption of the inoculum to the test item however could not be ruled out.

For comparison, RAC notes that the closely related RP 3:2 (Contram™ MBO and GrotaMar 71) has been shown in two tests to be not readily biodegradable.

RP 1:1, being an UVCB-substance, might contain constituents which are not sufficiently similar with regard to the property tested. Consequently, the degree of ultimate degradation (mineralisation to CO_2) of each of the various constituents and degradation products remains unknown in standard screening tests such as OECD 301D. It seems not possible to calculate a ThOD for RP 1:1 and a more careful consideration of the nitrification is recommended when measuring the COD. It is known that ready biodegradability tests may sometimes fail because of the stringent test conditions and that consistent positive test results should generally supersede negative test results. However, in the case of the UVCB substance RP 1:1 the borderline positive test result in one of the two OECD TG 301D tests may not be evaluated superseding the negative test. There is clear evidence from three tests, that RP 3:2 and RP 1:1 are not ready biodegradable and an adaptation of the inoculum cannot be ruled out. The weight of evidence approach by RAC incorporates that it has not been demonstrated for RP 3:2 and RP 1:1 that all constituents are sufficiently similarly degradable.

Based on the weight of the available evidence, including supporting data from RP 3:2, RAC concludes that RP 1:1 is not readily biodegradable. This is in line with the evaluation of RP 3:2.

Hydrolysis

It has been demonstrated in a laboratory test that RP 1:1 hydrolyses to formaldehyde and 2-hydroxypropylamine at rather low concentrations within a few hours. RAC notes that hydrolysis of RP 1:1 is rather the establishment of an equilibrium than irreversible hydrolysis. Consequently, the hydrolysis rate may not be taken as and abiotic degradation half-life as such. A more careful consideration of the hydrolysis is recommended. In addition, it has been demonstrated that hydrolysis of RP 1:1 is strongly dependent on its concentration in water and complete hydrolysis may only be assumed at

very low concentrations. The CLP guidance requires that hydrolysis has to be demonstrated under relevant environmental conditions. Since RP 1:1 is a UVCB substance, degradation may not follow single first order kinetics. Both degradation rate independent from concentration and degradation following first order kinetics, are required to extrapolate laboratory results to relevant environmental conditions (see guidance IR R.7b). At the 32th meeting of ECHA's Member State Committee (MSC-32) it was agreed that relevant environmental conditions include 12°C temperature. Although, the hydrolysis half-life DT_{50} under relevant environmental conditions (temperature, concentration, and pH) was not calculated, it may be reasonable to consider that the primary degradation half-life would be shorter than 16 days.

Rapid degradability

Following the guidance on the application of the CLP criteria (version 4.1, June 2015, II.2.3.8 Hydrolysis) to demonstrate rapid degradability data from hydrolysis studies could be considered "*only when it can be satisfactorily demonstrated that the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment*". While it has been demonstrated that RP 1:1 hydrolyses to the degradation products formaldehyde and 2-hydroxypropylamine it was questioned during PC if it has been sufficiently demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

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

For the hydrolysis product formaldehyde, ecotoxicity data have been assessed during the Biocides Review Programme in 2012 ("Formaldehyde Core Dossier"). All three trophic levels, fish, invertebrates and algae have been tested for acute aquatic toxicity. The sensitivity is at the same level, i.e. around 5.7 mg/L and above the CLP criteria to classify for aquatic acute hazard. However, no long-term study on fish is available. The algae study is only available as a literature publication without any raw data or concentration-response curves. Only the 72h E_rC_{50} of 5.7 mg/L was published. Consequently, the literature data does not allow the derivation of a NOE_rC, nor an E_rC_{10} or an E_rC_{20} and thus from this study no information on the chronic algae toxicity of formaldehyde is available. A second algae study was requested by several MSCA during the Biocides Review Programme and by one commenting MSCA during PC, but up to date has not been provided. For daphnia a NOEC of 1.04 mg/L was derived, which is close to the criterion (<1 mg/L) for classification.

There is evidence that formaldehyde is slightly more toxic than RP 1:1. Acute toxicity data show that fish are up to 23 times and invertebrates up to 5 times more sensitive to formaldehyde than to RP 1:1, while the sensitivity of algae is nearly identical. The chronic toxicity data for invertebrates (read-across to RP 3:2) show a slightly higher sensitivity to formaldehyde.

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

For the second relevant hydrolysis product 2-hydroxypropylamine an OECD assessment dated 2011 summarises acute ecotox data and QSAR estimations for all three trophic levels. The available information seems to indicate that 2-hydroxypropylamine to not fulfil the CLP criteria for aquatic acute toxicity. However, none of the available information was considered to be a reliable key study by the dossier submitter. No

additional information and none of the original study reports or scientific article were provided. Chronic toxicity of 2-hydroxypropylamine was not available in the CLH report. RAC concludes that the data do not sufficiently demonstrate that the hydrolysis product 2-hydroxypropylamine, does not fulfil the criteria for classification as hazardous to the aquatic environment.

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

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

Aquatic Bioaccumulation

RAC notes that a UVCB substance may only be considered to be one chemical substance for the purpose of assessing and testing the potential to bioaccumulate, if a clear case is made in the assessment for why all constituents are sufficiently similar with regard to the property tested. This has not been demonstrated for RP 1:1. RAC agrees with the dossier submitter that, although there are no experimental data about bioaccumulation available, in view of the rapid hydrolysis, it may be assumed that RP 1:1 does not fulfil the criteria on aquatic bioaccumulation.

Acute Toxicity

RAC agrees with the dossier submitter to not classify RP 1:1 as acutely hazardous to the aquatic environment (lower EC_{50} = 2.9 mg/L for *P. subcapitata*).

Chronic Toxicity

RAC agrees with the dossier submitter that the lowest chronic toxicity of RP 1:1 was derived for algae (*P. subcapitata*) with an E_rC_{10} of 0.148 mg/L. However, in contrast to the dossier submitter RAC considers RP 1:1 to be not rapidly degradable for the purpose of classification. This would result in a classification of RP 1:1 as Aquatic Chronic 2 (H411). RAC also applied the surrogate approach since adequate studies on chronic fish and invertebrate toxicity using RP 1:1 as test substance were not available. The surrogate approach results (the substance not rapidly degradable and the *Daphnia magna* EC_{50} of 29 mg/L) in a classification of RP 1:1 as Aquatic Chronic 3 (H412). Since the most stringent outcome should be chosen, **RAC concludes that RP 1:1 should be classified as Aquatic Chronic 2 (H411).**

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 +

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**
		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
A2.10/03	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 Lubrizol Deutschland GmbH S. Hahn, J. Regelmann, Fraunhofer Institute of Toxicology and Experimental Medicine, Department Chemical Risk Assessment, 24.7.2007 GLP not applicable, unpublished	Y	Lubrizol
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ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON REACTION PRODUCT OF PARAFORMALDEHYDE AND 2-HYDROXYPROPYLAMINE (RATIO 1:1); [HPT]

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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A3.1.2/01	2002	Determination of the Boiling Point of Contram 121. Kesla BioLab, Study No. KBL/2002/1176 BP, Mar. 2002 GLP, unpublished	Y	Lubrizol
A3.1.2/02	2000	Boiling Temperature of Grotan WS. Jai Research Foundation, Study No. 2685, Aug. 04, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.1.3/01	2002	Determination of the Relative Density of Contram 121. Kesla BioLab, Study No. KBL/2002/1176 RDI, Mar. 2002 GLP, unpublished	Y	Lubrizol
A3.1.3/02	2000	Relative Density of Grotan WS. Jai Research Foundation, Study No. 2686, Sep. 29, 2000 GLP, unpublished	Y	Schülke & Mayr
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
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
A3.2/02	2000	Vapour Pressure of Grotan WS. Jai Research Foundation, Study No. 2687, Nov. 06, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.2/03	2005	Estimation of physical-chemical properties of 3,3'- α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol using EpiSuite 3.12 GLP not applicable, published	N	Not applicable

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

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A3.4/01	2007	UV Spectrum of CONTRAM™ 121 [1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol, α,α',α"-trimethyl-] (CAS# 25254-50-6). Lubrizol Metalworking Additives, Spartanburg, SC, USA, July 3, 2007 No GLP, unpublished	Y	Lubrizol
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
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A3.4/06	2002	Analysenbericht SMN9701, Formaldehyd/Aminopropanol Kondensate – Aufkärung der Struktur. Spectral Service, 15.März 2002 No GLP not applicable, unpublished	Y	Schülke & Mayr
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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A3.7/01	2001	Fat Solubility of Grotan WS. Jai Research Foundation, Study No. 2690, Nov. 08, 2001 GLP, unpublished	Y	Schülke & Mayr
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A3.9	2002	Partition Coefficient (n-octanol/water) of Grotan WS. Jai Research Foundation, Study No. 3602, Jan. 08, 2002 GLP, unpublished	Y	Schülke & Mayr
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A3.10	1998	Sicherheitstechnische Überprüfung des Herstellprozesses von TPI 1600. Inburex GmbH, 26.05.1998 No GLP, unpublished	Y	Schülke & Mayr
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	2007a	Viscosity of Grotan WS, Schülke & Mayr, Research and Development 23.02.2006, Report (Dr. S. Hendrich) from 6.12.2007 No GLP, unpublished	Y	Schülke & Mayr
A3.14	2007b	Physical and chemical data of Grotan WS, Schülke & Mayr, Research and Development Jan. 10, 2007 No GLP, unpublished	Y	Schülke & Mayr
A3.16	2000	Oxidation Property of Grotan WS. Jai Research Foundation, Study No. 2695, Jul. 28, 2000 GLP, unpublished	Y	Schülke & Mayr
A3.17	2007	Reactivity towards container material: CONTRAM™ 121. Michael P. Scholz, Lubrizol, 19.07.2007 GLP not applicable, unpublished	Y	Schülke & Mayr + Lubrizol
A4.1/02	2007	Analytical method of determination the content of releasable formaldehyde of Grotan WS Schülke & Mayr, G.-D. Lembke, 18.12.2007 Non GLP, unpublished	Y	Schülke & Mayr
A4.2b	2008	Statement on the Vapour pressure of "α, α′, α″-Trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol: reaction product from paraformaldehyde and 2-hydroxypropylamine (ratio of 1:1)" (Hydroxypropyl-Triazin (HPT))". Dr.Stefan Hahn, Fraunhofer ITEM, 20 February 2008 GLP not applicable, unpublished	Y	Schülke & Mayr + Lubrizol
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A6.1.5/01	2001	OS157338, Skin sensitisation to the guinea-pig (Magnusson & Kligman method). Huntingdon Life Science Lt., Report No. LBL 045/004131/SS GLP, unpublished	Y	Schülke & Mayr + Lubrizol
A6.1.5/02	2001	Skin sensitisation study of Grotan WS in guinea pigs (guinea pig maximisation test). JAI Research Foundation., Study No. 2633 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
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A6.3.1/02	2002	Repeated dose 90-day oral toxicity study of Grotan WS in rats. JAI Research Foundation, India, Study No. 2636 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
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		GLP, unpublished		Lubrizol
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A7.1.1.2.1/01	2002	Study on the "Ready Biodegradability" of "Contram 121" according to OECD-Test Guideline 301D in the version of July 17th, 1992 (Closed-Bottle-Test). Institut Fresenius, Study No. IF-101/29362-00 GLP, unpublished	Y	Schülke & Mayr + Lubrizol
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A7.3.1	2005	Estimation of physical-chemical properties of 3,3'- α, α′, α″-trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol using EpiSuite 3.12 GLP not applicable, published	N	Not applicable
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8 ANNEXES

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